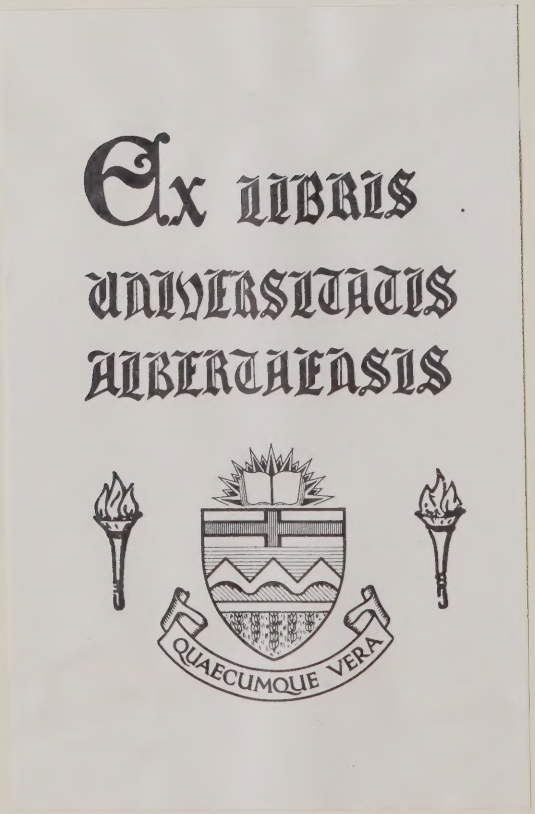

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FACTORS AFFECTING THE METABOLIZABLE ENERGY VALUE OF RAPESEED MEAL FOR GROWING CHICKENS AND LAYING HENS

by

© GOPI NATH LODHI

A THESIS

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ABSTRACT

Experiments were conducted to determine the metabolizable energy value of rapeseed meal for growing chickens and laying hens and to study factors which affect it. The factors studied included (1) the availability of its carbohydrate, (2) the absorbability of its protein and (3) the effect of the presence of isothiocyanates and (-)-5-vinyl-2-oxazolidinethione. For comparative purposes similar studies were conducted using soybean meal.

Results showed that the metabolizable energy value of nine samples of rapeseed meal averaged 1,203, 1,313 and 1,782 kcal per kilogram, respectively for four week old chicks, six week old chicks and laying hens when rapeseed meal was substituted weight for weight for glucose in a reference ration containing soybean meal. When rapeseed meal served as the sole source of dietary nitrogen, metabolizable energy values for four week old chicks, six week old chicks and laying hens were 1,880, 1,865 and 1,800 kcal per kilogram, respectively.

Determination of available carbohydrate, using a chick bioassay, showed that rapeseed meal contained 6.9% available carbohydrate. Using a chemical method, the available carbohydrate content of the same samples of rapeseed meal was 15.0%. Comparable values for soybean meal were 14.1 and 23.6%, respectively.

Results showed that the absorbability of protein in rapeseed meal for six week old chickens was 79.9%. Using colostomized hens, the average value obtained was 74.6%.

Comparable values for soybean meal were 85.4 and 80.8%, respectively. In all cases, values represent those obtained when the respective meal served as the sole source of protein in a semipurified ration.

Studies also showed that the presence of isothiocyanates and (-)-5-vinyl-2-oxazolidinethione in rapeseed meal had no adverse effect on its metabolizable energy value.

On the basis of these findings it may be concluded that rapeseed meal contains less metabolizable energy than soybean meal because it contains less available carbohydrate, less protein, and its protein has a lower absorbability than that in soybean meal.

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INTRODUCTION

Rapeseed meal, like other oilseed meals, is a potential source of protein for livestock and poultry. Studies have shown, that although rapeseed meal contains less protein than soybean meal, the distribution of essential amino acids in the protein moiety of rapeseed meal compares quite favorably with that of soybean meal. As a consequence, it has been found, in practical-type rations, that prepress-solvent and solvent-processed rapeseed meals are equivalent to soybean meal for chick growth promotion and feed conversion when energy-protein relationships are maintained constant. Only when over-heated expeller-processed rapeseed meal has been involved in such studies has slow growth and low feed conversion been reported.

Although a good deal of information on the quantity and quality of the protein in rapeseed meal is available, little has been reported on its content of metabolizable energy. Published results appear to indicate that the ability of the chick to utilize the energy of rapeseed meal is limited. Factors which may contribute to the low energy value of rapeseed meal when compared to soybean meal are: lower protein and higher fiber content of rapeseed meal as compared to soybean meal; differences in the availability of protein and carbohydrate in the two feedstuffs; and, the presence in rapeseed meal of thioglucosides, one of which on hydrolysis by the enzyme myrosinase yields goitrin ((-)-5-vinyl-2-oxazolidinethione), the others volatile isothiocyanates.

The following studies were undertaken to determine the metabolizable energy value of rapeseed meal for growing chickens and laying hens and to study factors that might affect its metabolizable energy content.

METABOLIZABLE ENERGY VALUE OF RAPESEED MEAL FOR GROWING CHICKENS AND LAYING HENS

Review of Literature

Partition of Feed Energy

The parameters of caloric evaluation of feedstuffs have conventionally been their content of gross energy, digestible energy, metabolizable energy and net or productive energy. The relationship between these parameters is shown diagrammatically in Figure 1.

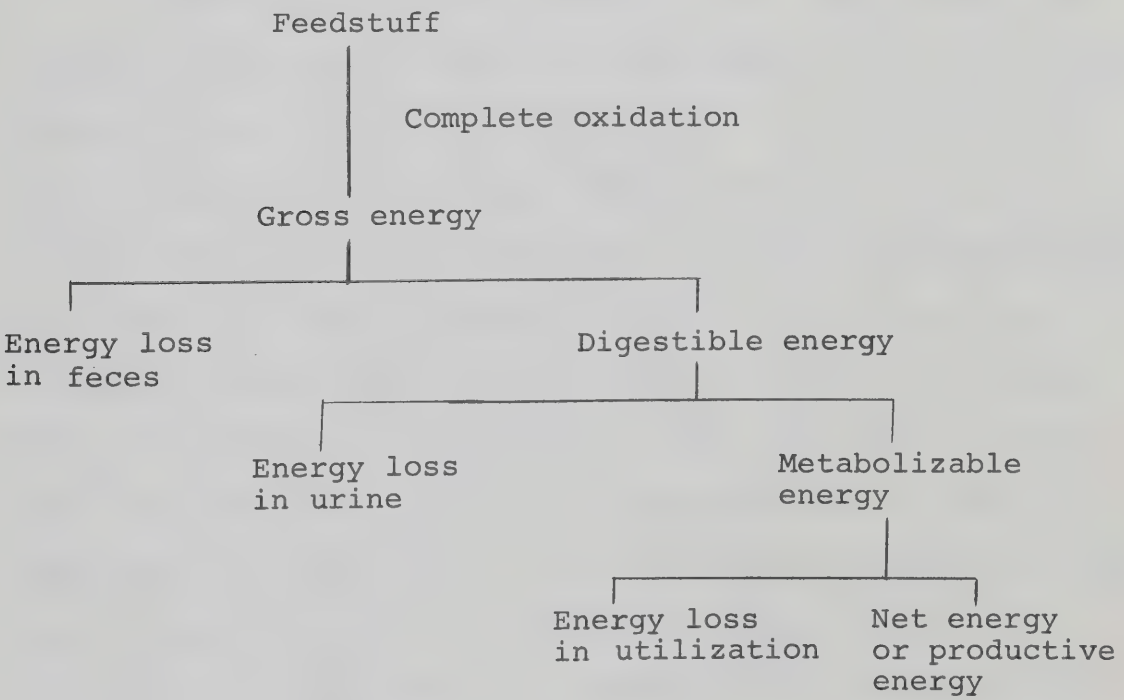


FIGURE 1. Partition of feed energy

Net energy should be the most useful measure of feed energy because it takes into account all energy losses due to digestion and metabolism and thus measures the maximum amount of energy available for useful work. It is, however, the measure most subject to variation and most difficult to determine. It is influenced by the level of feed intake, the balance of nutrients in the ration, the type of animal used in the determination and the environmental conditions to which the animals are subjected during the determination (Hill and Anderson, 1958).

Metabolizable energy measures the total energy of the feed available for metabolic processes. It is the gross energy of the feed minus the energy lost in the feces and urine. Metabolizable energy determinations are particularly well suited for avian species since in birds undigested feed and urine are voided together. Studies have shown that, in the fowl, metabolizable energy values of feeds are essentially unaffected by level of feed intake and rate of growth (Hill and Anderson, 1958), egg production (Hill, 1965), breed and sex (Begin, 1967), and balance of nutrients in the ration (Carew and Hill, 1961; Sibbald et al., 1962). Furthermore, except where the determination involves fat, age of the bird has little effect on the values obtained (Renner and Hill, 1960b). Moreover, the additional correction for a state of nitrogen equilibrium proposed by Hill and Anderson (1958) to correct for variations in the protein deposited due to differences in feeding regimen has brought

about a greater uniformity in metabolizable energy values. This greater uniformity, despite variations in experimental conditions, together with the relatively high precision with which metabolizable energy values can be determined, have made their use preferable to productive energy values.

The limitations of digestible energy and gross energy as measures of energy utilization are obvious. The first does not take into account the energy lost in the urine while the latter ignores energy lost in both feces and urine.

Determination of Metabolizable Energy

The determination of metabolizable energy using chickens has been carried out in several laboratories (Fraps and Carlyle, 1942; Carpenter and Clegg, 1956; Hill and Anderson, 1958; Matterson et al., 1958; Potter and Matterson, 1960; Sibbald et al., 1960; Sibbald and Slinger, 1963a). The method followed has varied from laboratory to laboratory.

One procedure, used by Matterson et al. (1958), is to feed the test material alone or in combination with adequate amounts of vitamins and minerals and to determine its metabolizable energy value directly. This method is open to criticism since the ration contains either an excessive or inadequate amount of several nutrients and its utilization may be abnormal, particularly if fed over prolonged periods of time.

A second procedure which has been used extensively by Hill and Anderson (1958) and by Potter and Matterson

(1960) consists of feeding a basal ration containing a chemically pure material of predetermined metabolizable energy content and a test ration, similar to the basal ration, but in which a portion of the reference material is replaced by the feedstuff to be assayed. The metabolizable energy content of the two rations are determined and the metabolizable energy content of the material under test is calculated. This method assumes a constant metabolizable energy value for the reference material. As in the previously referred to method, the test ration may be criticized on the grounds that it may contain an excessive amount of several nutrients.

A third procedure for determination of metabolizable energy is that of Sibbald et al. (1960) and Sibbald and Slinger (1963a). It involves feeding a practical-type control ration and a test ration formulated by replacing part of the control ration with the ingredient to be assayed. The metabolizable energy values of the two rations are determined and the metabolizable energy value of the test material is then calculated. This procedure may be criticized on the grounds that the test ration may be nutritionally unbalanced and the calculations assume a constant metabolizable energy value for each unit of control ration replaced by test material. It has the advantage, however, that a true control is included with each set of determinations.

Factors Affecting Metabolizable Energy Values

Of major importance is whether the balance of nutrients in the ration affects the metabolizable energy values obtained. In this regard, it should be noted, that in assessing the metabolizable energy content of protein-rich feedstuffs of vegetable origin, the principle difference between the basal or control ration and the test ration is in the level of protein, assuming, of course, that constant levels of minerals and vitamins are added to each ration.

Sibbald et al. (1962) determined the metabolizable energy content of wheat, barley and corn when all or part of a basal mix was replaced by the grain under study. The protein contents of the rations containing wheat, barley and corn varied from 14 to 33%, 13 to 31% and 8 to 31%, respectively. The metabolizable energy values for the grains were found to be unaffected by protein level.

Olson et al. (1961) determined the metabolizable energy content of soybean meal and meat meal when each of these meals replaced one-third or all of two test rations that contained 16 or 33% protein. Results obtained showed that the metabolizable energy value for soybean meal was unaffected by either dietary protein level or level of substitution. However, the metabolizable energy value for meat meal was reduced when either the protein level of the reference ration or the percentage of meat meal in the ration was increased. Sibbald and Slinger (1962) measured the metabolizable energy content of soybean meal and meat meal

when incorporated at three levels; 25, 50 and 75% of the ration. The protein contents of the rations varied from 20 to 38%. Results obtained indicated that the metabolizable energy content of soybean meal was unaffected by level of inclusion in the ration, whereas the metabolizable energy value of meat meal was increased with increasing levels of dietary inclusion. The reason for these conflicting results with respect to meat meal is not apparent.

In studies involving feeding of different levels of casein, Anderson (1955) found that the metabolizable energy value of casein was unaffected by the protein content of the ration.

The effect of quality of protein rather than quantity of protein on metabolizable energy values has received the attention of several workers. Carew and Hill (1961) and Sibbald and Slinger (1962) have shown that supplementation of soybean meal with methionine does not affect its metabolizable energy value. Hill (1964) has reported that the metabolizable energy value of sesame meal is unaffected by lysine supplementation. Support for the contention that amino acid balance has little direct effect on the metabolizable energy of a feedstuff is given by the finding of Sibbald and Slinger (1963b) that the metabolizable energy values of protein mixtures were equal to the sum of the metabolizable energy of their parts.

Metabolizable Energy Content of Rapeseed Meal for Poultry

Studies on the metabolizable energy content of rapeseed meal are limited. Sibbald and Slinger (1963b), using chicks, reported a value of 1,670 kcal of metabolizable energy per kilogram (dry matter basis) for one sample of solvent-processed rapeseed meal which analyzed 43.1% protein (N x 6.25). On the other hand, Sell (1966), using hens, obtained a value of 2,290 kcal of metabolizable energy per kilogram for one sample of solvent-processed rapeseed meal which analyzed 38.3% protein. In digestibility trials with laying hens in which the urinary and digestive tracts had been operatively separated, Kubota and Morimoto (1965) reported a total digestible nutrient value for one sample of rapeseed meal of 41.7%. This value is low when compared to the value of 65.7% for soybean meal reported for adult chickens by Bolton (1957). These results suggest that the metabolizable energy value of rapeseed meal for adult chickens should be about two-thirds that of soybean meal.

Studies on the digestibility of the energy in rapeseed meal by rats and pigs have been reported by Hussar and Bowland (1959) and Manns and Bowland (1963). These workers found that the inclusion of 10% of rapeseed meal in the ration of the rats in place of soybean meal, on an isonitrogenous basis, significantly reduced the apparent digestible energy. No consistent trend in this regard was noted when pigs were the experimental animals. Later Bowland and Schuld (1968) reported that the inclusion of

8% rapeseed meal in starting and growing rations for pigs as a replacement for soybean meal on an isonitrogenous basis had no adverse effect on the metabolizable energy value of the ration. Whether these findings reflect a species difference in the utilization of energy from rapeseed meal is difficult to judge on the basis of the data available.

Studies at the University of Alberta

The purpose of this study was two-fold: firstly, to gather more data on the metabolizable energy value of rapeseed meal for chicks and hens; secondly, to determine whether or not metabolizable energy values obtained for rapeseed meal are affected by the length of time that the birds have received rapeseed meal prior to the time at which the metabolizable energy determinations are made. The latter seemed advisable since the hypothyroidism caused by the inclusion of rapeseed meal in the diet of chicks (see Part II), which according to Clandinin et al. (1966) is corrected by the chick in a period of about three weeks, could conceivably affect metabolizable energy values obtained.

Experimental

Nine samples of rapeseed meal were used in the study; five were prepress-solvent processed (meals 1, 3, 6, 8, 9) while four were solvent-processed (meals 2, 4, 5 and 7). Proximate composition, as determined by A.O.A.C. methods

(1960); and oxazolidinethione and isothiocyanate content, as determined by methods developed by Astwood et al. (1949) and Wetter (1955, 1957) are given in Table 1.

The metabolizable energy values were determined by the method of Hill and Anderson (1958). Reference rations used for chicks and hens are shown in Table 2. Test rations were formulated by replacing 30 parts of glucose or sucrose in the respective test rations with 30 parts of rapeseed meal, all substitutions being made on a dry matter basis.

In the determination of the metabolizable energy of rapeseed meal for chicks, male and female crossbred (Dominant White male x White Plymouth Rock female) chicks were used. They were maintained in electrically heated, thermostatically controlled battery brooders, with raised wire screen floors, in a temperature-controlled laboratory. In one experiment, equal numbers of male and female chicks were randomly allotted to groups of 10 chicks each at one day of age. In two experiments, they were reared to one week of age on the reference ration and then equal numbers of male and female chicks were allotted to groups of 10 chicks each on the basis of body weight, equalizing both mean weight and weight distribution among the groups. Each experimental ration was fed to two duplicate lots from either 0 to 42 or 7 to 42 days of age and to two duplicate lots from 21 to 42 days of age. Chicks receiving the rapeseed meal-containing rations from 21 to 42 days of age were maintained on the reference ration for the first 21 days. Feed and water were supplied

TABLE 1. - Composition of rapeseed meals[1]

Rapeseed meal	Moisture	Protein	Fat	Fiber	Ash	Isothio-cyanate	Oxazolidine-thione
No.	%	%	%	%	%	mg /g	mg /g
1	10.9	36.8	2.7	12.5	6.5	4.2	1.8
2	9.2	35.4	2.5	13.2	6.5	4.2	1.7
3	12.8	33.9	3.4	13.3	7.0	4.2	1.7
4	9.9	36.0	2.2	13.2	6.2	3.1	3.4
5	11.3	37.5	2.2	12.5	6.2	4.3	1.7
6	12.6	35.1	1.7	13.3	6.5	4.2	1.9
7	9.9	36.4	2.5	14.0	6.4	2.4	1.6
8	9.3	36.3	2.5	13.4	6.5	3.4	1.4
9	10.0	35.9	1.6	13.3	6.7	3.8	1.2
Averages	10.8	35.9	2.4	13.2	6.9	3.8	1.8

[1]Values are expressed on an air-dry basis.

TABLE 2. - Composition of reference rations

Ingredients	Rations	
	Chicks	Hens
	%	%
Glucose (Cerelose)	50.485	
Sucrose		51.67
Soybean meal (48.5% protein)	35.0	32.0
Cellulose		5.0
Glycine	1.0	
DL-methionine	0.5	0.1
Dried brewer's yeast	2.5	
Dried whey	2.0	
Mineral mixture	4.91[1]	7.9[2]
Vitamin mixture	0.58[3]	0.32[4]
Soybean oil	2.0	2.0
Antioxidant (Ethoxyquin)	0.025	0.01
Chromic oxide mixture[5]	1.0	1.0

[1]Supplied in milligrams per 100 grams ration: $CaHPO_4$, 2600; $CaCO_3$, 1300; NaCl, 600; K_2HPO_4, 220; $MgSO_4$, 115; $FeSO_4 \cdot 7H_2O$, 28; $MnSO_4 \cdot H_2O$, 33.5; $ZnCO_3$, 9.7; $CuSO_4 \cdot 5H_2O$, 0.78; KI, 0.29; and Na_2SeO_3, 0.022.

[2]Supplied in milligrams per 100 grams ration: $CaHPO_4$, 4000; $CaCO_3$, 3000; NaCl, 500; K_2HPO_4, 220; $MgSO_4$, 120; $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 16.8; $ZnCO_3$, 9.25; $CuSO_4 \cdot 5H_2O$, 1.5; KI, 0.33; and Na_2SeO_3, 0.02.

[3]Supplied per 100 grams ration: thiamine, 1.0 mg; riboflavin, 1.0 mg; calcium pantothenate, 4.0 mg; biotin, 0.04 mg; pyridoxine, 2.0 mg; niacin, 8.0 mg; folacin, 0.3 mg; menadione, 0.3 mg; vitamin B_{12}, 0.5 µg; choline chloride, 0.3 g; vitamin A, 1000 IU; vitamin D_3, 150 ICU; vitamin E, 3.3 IU; and aureomycin, 1.0 mg.

[4]Supplied per 100 grams ration: thiamine, 1.0 mg; riboflavin, 2.0 mg; calcium pantothenate, 5.0 mg; niacin, 15.0 mg; folacin, 0.2 mg; pyridoxine, 1.5 mg; biotin, 0.05 mg; vitamin A, 1000 IU; vitamin D_3, 75 ICU; and vitamin E, 1.1 IU.

[5]Contained: 30% chromic oxide, 70% flour.

ad libitum. The chicks were weighed at weekly intervals and feed consumption was determined weekly.

Excreta were collected at 24 hour intervals at 26, 27 and 28 days of age and again at 40, 41 and 42 days of age. Each three-day collection was maintained in the frozen state until processed. Chromic oxide was incorporated in all rations at a level of approximately 0.3% as an index substance in order to eliminate the need for quantitative collections of excreta and quantitative measurements of feed intake. The methods of conducting chemical analyses for moisture, nitrogen, combustible energy and chromic oxide and of computing metabolizable energy from these data have been described previously (Hill and Anderson, 1958; Hill et al., 1960). Glucose, the reference material used in the rations for chicks, was assumed to have a metabolizable energy value of 3.64 kcal per gram (Anderson et al., 1958).

At six weeks of age, the female chicks in each group were killed, both thyroids were removed and weighed and thyroid-to-body-weight ratios were calculated.

In the determination of the metabolizable energy of rapeseed meal for hens, duplicate groups of four White Plymouth Rock hens, approximately 15 months old, were used. They were allotted to their respective experimental groups on the basis of body weight, equalizing both mean body weight and weight distribution among the groups. They were fed the experimental rations for 28 days. Fecal collections were made on the 6th, 7th and 8th and again on the 26th,

27th and 28th day of the experiment. The methods of processing excreta, conducting chemical analyses and computing metabolizable energy from these data were similar to those used for chicks. Sucrose, the reference material used in the rations for hens, was assumed to have a metabolizable energy value of 3.80 kcal per gram (Hill, 1962).

Results and Discussion

Summarized in Table 3 are data showing metabolizable energy values of the rapeseed meals for chicks determined during the 4th week of life after the chicks were fed rapeseed meal-containing diets for either 5 or 19 days. Analysis of variance (Steel and Torrie, 1960) showed that the rapeseed meals differed significantly in energy content ($P < 0.01$) and that the length of time that chicks were fed the rapeseed meal-containing diets prior to the determination had small but significant ($P < 0.05$) effects on metabolizable energy. The data show the average metabolizable energy content was 1,104 and 1,203 kcal per kilogram, respectively, for the rapeseed meals fed for 5 and 19 days prior to the determination.

Also summarized in Table 3 are data showing metabolizable energy values of the rapeseed meals determined during the 6th week of life after chicks had been fed rapeseed meal-containing diets for either 19 or 33 days. Analysis of variance (Steel and Torrie, 1960) showed that the rapeseed meals differed significantly ($P < 0.01$) in

TABLE 3. - Effect of age and duration of feeding rapeseed meal-containing rations on the metabolizable energy value[1] of rapeseed meal for chicks and hens

Rapeseed meal	Chicks				Hens	
	Interval on R.S.M., days		Interval on R.S.M., days		Interval on R.S.M., days	
	21 to 28	7 to 28[2]	21 to 42	7 to 42[2]	1 to 8	1 to 29
No.	kcal/kg	kcal/kg	kcal/kg	kcal/kg	kcal/kg	kcal/kg
1	847[3]	1,298	1,434	1,478	1,102	1,945
2	1,016	1,221	1,131	1,426	1,564	1,646
3	1,104	1,327	1,355	1,355	1,316	1,929
4	979	854	895	1,093	1,382	1,725
5	1,239	1,338	1,549	1,522	1,373	1,531
6	1,135	1,096	1,236	1,302	1,243	1,553
7	1,109	1,142	1,137	1,023	1,272	1,690
8	1,038	1,065	1,096	1,126	1,362	1,984
9	1,470	1,483	1,582	1,489	1,404	2,031
Averages	1,104	1,203	1,269	1,313	1,335	1,782

[1]Collections for metabolizable energy determinations were made on the last 3 days of each experimental period.

[2]Rations containing rapeseed meals (R.S.M.) 1, 5 and 6 were fed from 1 day of age.

[3]Values are expressed on a dry matter basis and represent the average of duplicate groups.

metabolizable energy content; however, the length of time that chicks were fed the rapeseed meal-containing rations prior to the determination did not affect energy value significantly ($P < 0.05$). Since Clandinin et al. (1966) have shown that chicks compensate for the goitrogenic effect of rapeseed meal in a period of about 21 days, the adverse effects which hypothyroidism might have on absorbability and/or digestion at 5 days could have disappeared by 19 days.

The effect that hypothyroidism has on overall absorbability of nutrients has not been reported. However, Althausen and Stockholm (1938) showed that thyroidectomy decreased rate of absorption of glucose and galactose by 43%. The possibility that hypothyroidism caused by feeding rapeseed meal might reduce digestion is suggested by the finding of Sun et al. (1954) that gastric secretion is reduced in rats made hypothyroid by the administration of radioiodine or by feeding thiouracil. Whether the goitrogen in rapeseed meal affects overall absorbability and whether the observed increase in metabolizable energy with length of feeding is due to thyroid compensation awaits further study.

Statistical treatment of the metabolizable energy values obtained during the 4th and 6th weeks of life showed that the metabolizable energy value of rapeseed meal for six week old chicks was slightly but significantly ($P < 0.01$) higher than for four week old chicks. These results suggest

that the ability of the chick to utilize rapeseed meal improves with age. It will be noted that the average value for six week old chicks is considerably lower than that reported (1,670 kcal per kg) by Sibbald and Slinger (1963b). However, it should be noted that the meal that Sibbald and Slinger studied contained 43.1% protein and probably around 11% fiber whereas the meals studied herein averaged 35.9% protein and 13.2% fiber (see Table 1).

Metabolizable energy values of the rapeseed meals for hens are also given in Table 3. Analysis of variance (Steel and Torrie, 1960) showed that the metabolizable energy value of the rapeseed meals was significantly higher ($P < 0.01$) after hens were fed the rapeseed meal-containing rations for 26 days than for only five days. These results are similar to those observed with chicks during the 4th week of life, but are at variance with the findings of Sell (1966) that the metabolizable energy value of rapeseed meal for hens was the same irrespective of whether rapeseed meal was fed for 10 or 20 days before the determination was made. However, if hypothyroidism actually affects metabolizable energy values, it would seem probable that after 10 days of feeding rapeseed meal major adjustment in thyroid activity would already have taken place and as a consequence one perhaps should not expect the results of the two studies to agree.

The average value of 1,782 kcal of metabolizable energy per kilogram dry matter observed for hens is somewhat

lower than the value of 2,290 kcal per kilogram dry matter reported by Sell (1966). Whether the difference in values obtained is due to some difference in the method of determination or to variance in the quality of the meals studied is not known. However, it should be pointed out that the average value for hens found in this study agrees well with the value that could be estimated from the digestible nutrients in rapeseed meal as reported by Kubota and Morimoto (1965).

Comparison of the metabolizable energy values obtained with chicks and hens showed that hens were better able to utilize rapeseed meal than were chicks at either four or six weeks of age. The values observed for four week old chicks, six week old chicks and hens after feeding rapeseed meal for at least 21 days were 1,203, 1,313 and 1,782 kcal per kilogram. Previous studies have shown that the ability of the chick to digest and absorb certain feedstuffs increases with age. For example, Renner and Hill (1960a) and Hill and Renner (1963) observed that the metabolizable energy of over-heated soybean flakes for chicks and hens was 2,024 and 2,552 kcal per kilogram, respectively. Renner and Hill (1960b) also observed that the ability of the chick to utilize tallow increased with age. In contrast, quantitative comparisons between young chicks and adult hens showed similar values for commercially produced dehulled soybean meal, corn oil and lard.

Data on growth and thyroid size collected during

the experiments with chicks are shown in Table 4. Analysis of variance of the growth data in each experiment showed that substitution of 30 parts rapeseed meal for 30 parts glucose in the reference ration had no significant effect on growth. Similar treatment of the data on thyroid-to-body-weight ratios showed that of the nine rapeseed meals fed for 35 or 42 days, meals 3, 4, 7, 8 and 9 increased thyroid-to-body-weight ratios significantly ($P < 0.05$) over those of chicks fed the reference ration. In the case of the groups fed rations containing rapeseed meal for 21 days, meals 4, 7, 8 and 9 caused significant increased in thyroid-to-body-weight ratios.

In order to determine whether energy utilization was affected, caloric efficiencies were calculated at the initiation of feeding of rations containing rapeseed meal (see Table 4). Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data obtained during the 4th week of life indicated that chicks fed rations containing rapeseed meal prior to the 4th week of life utilized energy less efficiently ($P < 0.05$) than chicks in which the feeding of rapeseed meal was commenced at the beginning of the 4th week. This difference in caloric efficiency may be due to changes in secretion of the thyroid gland. This concept is supported by the finding that by the 6th week of life when all chicks should have compensated, differences in caloric efficiency had disappeared. In this regard caloric efficiencies during the

TABLE 4. - Body weight, thyroid size and caloric efficiency of chicks fed rations containing rapeseed meal

Ration	Weight, 6 weeks		Thyroid size, 6 weeks		Calories consumed per gram gain during the 4th week	
	Interval on R.S.M., days		Interval on R.S.M., days		Interval on R.S.M., days	
	21 to 42	7 to 42[1]	21 to 42	7 to 42[1]	21 to 28	7 to 28
	grams[2]		mg/100 g body wt[3]		kcal	
Reference		825		10.22		5.98
R.S.M. 1	754	738	12.58	17.50	4.57	5.39
R.S.M. 6	810	744	13.67	19.27	4.85	5.00
R.S.M. 5	801	735	14.80	20.73	4.76	5.50
Reference		894		10.42		5.89
R.S.M. 2	843	846	20.1	18.6	5.10	5.76
R.S.M. 3	852	845	14.6	22.5	4.99	5.81
R.S.M. 4	872	862	31.7	39.7	5.11	5.62
Reference		888		10.00		5.42
R.S.M. 7	841	860	20.0	20.0	4.72	5.06
R.S.M. 8	876	857	16.8	20.4	4.69	4.90
R.S.M. 9	880	886	15.8	16.8	4.74	5.28

[1]Rations containing rapeseed meals (R.S.M.) 1, 5 and 6 were fed from 1 day of age.

[2]Values are averages of duplicate groups.

[3]Values are averages of 10 female chicks (5/replicate group).

6th week were 6.88 and 6.77 kcal consumed per gram gained for chicks fed rapeseed meal for 21 and 35 days respectively.

Summary

A study was made on the metabolizable energy value of rapeseed meal for chicks and hens and on whether or not metabolizable energy values obtained on rapeseed meal are affected by the length of time that the birds have received rapeseed meal prior to the time at which the metabolizable energy determinations are made.

Results showed that the average metabolizable energy content of nine samples of prepress-solvent and solvent-processed rapeseed meal was 1,203, 1,313 and 1,782 kcal per kilogram, respectively, for four week old chicks, six week old chicks and hens fed diets containing 30% rapeseed meal for at least 21 days. These results show that the ability of the chicken to utilize rapeseed meal increases with age.

Results also showed that the metabolizable energy content of rapeseed meal for four week old chickens and hens increased when the measurement was made after chicks and hens had been fed rapeseed meal for 26 and 19 or 26 days, respectively, rather than for only five days. The possibility exists that the adverse effects which hypothyroidism might have on absorbability, while present at five days, could have disappeared by 19 or 26 days since by this time compensating changes would have occurred in the thyroid glands of the chicken.

PART II

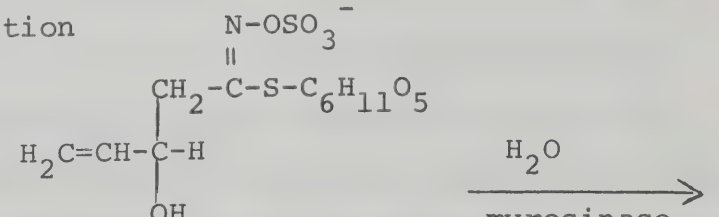
EFFECT OF GOITROGEN(S) IN RAPESEED MEAL ON METABOLIZABLE ENERGY

Review of Literature

Presence of Goitrogen(s) in Rapeseed and Rapeseed Meal

The goitrogenicity of rapeseed has been reported by Kennedy and Purves (1941) and of rapeseed meal by Blakely and Anderson (1948). The principal goitrogen in rapeseed has been isolated and identified by Astwood et al. (1949) and Carroll (1949) as L-5-vinyl-2-thiooxazolidone and called goitrin. More recently, goitrin has been assigned the chemical name (-)-5-vinyl-2-oxazolidinethione (Greer, 1962). Goitrin has been shown to occur in rapeseed as a thiglucoside (progoitrin) which on hydrolysis by the enzyme myrosinase yields glucose, bisulfate and 2-hydroxy-3-butenyl isothiocyanate (Greer, 1956; Virtanen, 1965; VanEtten et al., 1969). The latter compound is unstable and cyclizes to the antithyroid substance, (-)-5-vinyl-2-oxazolidinethione (Kjaer, 1960). The two reactions involved are as follows:

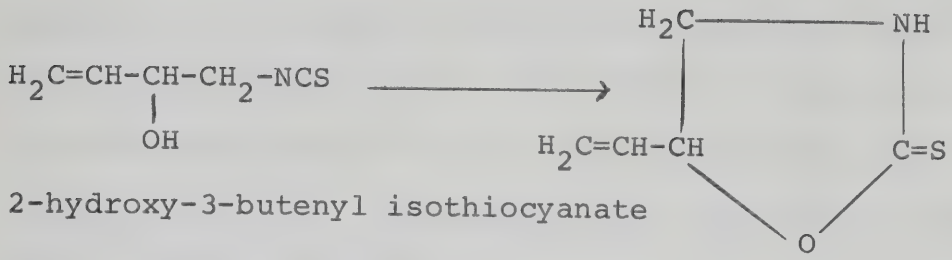
First reaction



(progoitrin)

glucose bisulfate 2-hydroxy-3-butenyl isothiocyanate

Second reaction



The part, if any, that volatile isothiocyanates and thiocyanates, released by the action of myrosinase on other glucosides in rapeseed (Virtanen, 1965; VanEtten et al., 1969) play in the goitrogenicity of rapeseed and rapeseed meal is unknown. However, their significance should not be completely discounted, since thiocyanate has been implicated in "cabbage goitre" (Webster et al., 1931).

Levels of Goitrogen(s) in Rapeseed and Rapeseed Meal

The levels of thioglucosides in defatted rapeseed have been shown to be related to the variety of rapeseed and the environmental conditions under which the seed is grown. Wetter and Craig (1959) reported that the isothiocyanate content varied from 4.85 to 5.36 and 4.33 to 4.91 mg per gram of defatted seed produced from Brassica campestris (Polish-type) and Brassica napus (Argentine-type) seed, respectively. Oxazolidinethione content varied from 1.33 to 1.55 and 5.31 to 5.60 mg per gram of defatted seed, respectively, for these two types of rapeseed. Similar findings were reported by Clandinin et al. (1959). In addition, the

latter workers found that the oxazolidinethione content appeared to be affected by the environmental conditions under which the seed was grown. The effect of fertilization with sulfate fertilizers on the isothiocyanate and oxazolidinethione content of rapeseed has been reported by Downey and Wetter (1964). They found that the response to sulfur fertilizers was much less for B. campestris than for B. napus. The increase, associated with fertilizer treatment, was about four times for isothiocyanate and two times for oxazolidinethione.

In the production of rapeseed meal from rapeseed by the prepress-solvent and solvent methods of processing, it is customary to raise the temperature of the seed or crushed seed to 100°C as quickly as possible (Youngs, 1965). This is done to destroy the myrosinase in the seed and thus prevent it from liberating goitrin and isothiocyanates from their glucoside precursors during the extraction process. As a consequence of the destruction of myrosinase early in the processing procedure, most of the potential goitrogenic activity remains in precursor form which in the absence of an intrinsic or exogenous source of myrosinase has little antithyroidal effect. However, conversion of some of the progoitrin in rapeseed meal to goitrin in the gastrointestinal tract as a result of intrinsic myrosinase produced by intestinal microorganisms (Gmelin and Virtanen, 1959; Greer and Deeney, 1959) has been shown to occur. This, plus the possibility of myrosinase getting into mixed feeds as a

result of use of grains contaminated with mustard seed, a rich source of myrosinase (Gaines and Goering, 1960), probably account for the mild goitrogenicity of rapeseed meals which contain goitrogen(s) only in precursor form(s).

Effects of Goitrogen(s) in Rapeseed Meal on Thyroid Size, Thyroid Function and Growth Rate

Numerous workers (Witz et al., 1950; Dow and Allen, 1954; Klain et al., 1956; Clandinin et al., 1959) have reported thyroid enlargement as a result of feeding rapeseed meal to poultry. In general, meal produced from Brassica napus (Argentine-type) seed has been shown (Klain et al., 1956; Clandinin et al., 1959) to cause a greater degree of thyroid enlargement than meal produced from Brassica campestris (Polish-type) seed. This result is in agreement with the previously referred to higher level of goitrin in seed of the B. napus variety as compared to the level of goitrin in seed of the B. campestris variety.

Clandinin and Bayly (1960), in their study on the histology of the thyroid glands of growing chickens and laying hens that had been fed rapeseed meal with and without stabilized iodine for a month or more, found that an increase in number and size of epithelial cells in the glands accounted for the increase in thyroid size in growing chickens fed rapeseed meal. In the case of laying hens fed rapeseed meal, initially the glands exhibited enlargement as a result of an increase in the number of follicles, the follicles being well defined. As time on treatment

progressed, the follicles toward the central portion of the glands became distorted and completely filled with cells and the amount of colloid was greatly reduced. When stabilized iodine was added to the ration of growing chickens and laying hens, the glandular enlargement was found to be caused by increased follicle size and increased storage of colloid; however, the cells appeared more normal in size and shape.

Recently, Clandinin et al. (1966) studied the effect of feeding synthetic goitrin, (±)-5-vinyl-2-oxazolidinethione, on the uptake and release of radioiodine from the thyroid glands. It would appear, from their studies, that the early effects of feeding goitrin to chicks include suppression of iodine uptake by the glands and a reduction in the secretion of thyroxine from the glands. However, after three or four weeks of feeding a ration containing the goitrogen, it would seem that compensatory changes occur in the thyroid glands which permit a return to normal rates of thyroxine secretion. Similar conclusions may be drawn from the work of Matsumoto et al. (1968) which involved the feeding of natural goitrin, (-)-5-vinyl-2-oxazolidinethione, to chicks.

The isothiocyanates and (-)-5-vinyl-2-oxazolidine-thione, liberated by myrosinase from their parent thio-glucosides in rapeseed meal, are known to act differently on the thyroid gland at the cellular level (Greer et al., 1964). The goitrogenesis caused by isothiocyanates may be similar to "cabbage goitre", a condition caused by competitive inhibition of iodine uptake by the thyroid in the presence

of thiocyanate. On the other hand, the goitrogen(s) in rapeseed have been shown by Griesbach and Purves (1943) and Purves (1943), in work with rats, to interfere with the power of the thyroid to synthesize thyroxine. These workers showed that the resultant fall in the level of thyroxine in the circulation stimulated the pituitary to secrete excessive amounts of thyrotropin which in turn caused the thyroid to undergo hyperplasia and hypertrophy. By the end of three weeks on rations containing rapeseed, thyroid changes were at a maximum. After this, growth of the glands paralleled that of growth of the rats. The thyroids had reached physiological equilibrium at an increased thyroid-to-body-weight ratio. Studies by Matsumoto et al. (1969) have shown high levels of monoiodotyrosine in the thyroid glands of chicks fed (-)-5-vinyl-2-oxazolidinethione and suggested that this goitrogen inhibits the formation of diiodotyrosine which is necessary for the production of thyroxine.

In addition to goitrogenesis, growth depression as a consequence of feeding (±)-5-vinyl-2-oxazolidinethione has been reported (Clandinin et al., 1966). These workers found that the addition of 0.15% synthetic goitrin to the ration of chicken reduced growth rate. However, the level of goitrin added was considerably higher than would be encountered under practical feeding conditions, even under circumstances where an exogenous source of myrosinase was present to release goitrin from its precursor in rapeseed meal. More recently, Matsumoto et al. (1968) have reported

no deleterious effect on the growth of chicks as a result of feeding 0.05% (-)-5-vinyl-2-oxazolidinethione.

Effect of Goitrogens on Metabolizable Energy

The effects of hypothyroidism on absorption of specific nutrients and on the secretion of digestive enzymes have been studied. Althausen and Stockholm (1938) and Scow and Foglia (1951) found that, in rats, injection of thyroxine increased the rate of absorption of glucose and galactose, while thyroidectomy decreased the absorption of these nutrients. Similar effects were noted by Althausen (1949) with respect to oleic acid. Numerous workers (Fink, 1944; Russell and Nassett, 1953; Sun et al., 1954) have reported a significant depression in gastric and intestinal secretion in monogastric animals after they had been made hypothyroid by total thyroidectomy or by the administration of radioactive iodine or thiouracil. No work, however, has been reported on the effect of hypothyroidism on overall absorbability.

Studies at the University of Alberta

The objective of the following studies was to determine whether synthetic goitrin, natural goitrin or thiocyanate as supplied by potassium thiocyanate affects the metabolizable energy value of a ration.

Experimental

In Experiment 1, chicks were fed the soybean meal reference ration (Part I, Table 2) supplemented with 0, 0.045 and 0.090% of (±)-5-vinyl-2-oxazolidinethione (± OT) which was synthesized in our laboratory from butadiene monoxide in the manner described by Ettlinger (1950).

In Experiments 2 and 3, chicks were fed the soybean meal reference ration (Part I, Table 2) and rations containing 30% rapeseed meal, with, and without, 1% ground mustard seed. The rapeseed meal rations were formulated from the reference ration by replacing 30 parts of glucose with 30 parts rapeseed meal, all substitutions being made on a dry matter basis. Ground mustard seed was added to serve as a source of the enzyme, myrosinase, which is needed for the conversion of progoitrin in rapeseed meal to goitrin. Analysis (Astwood et al., 1949; Wetter, 1957) showed that the rapeseed meals used in Experiments 2 and 3 contained 0.18 and 0.23% (-)-5-vinyl-2-oxazolidinethione (- OT), respectively.

In Experiment 4, chicks were fed the soybean meal reference ration and the reference ration supplemented with 0.09% potassium thiocyanate.

In all experiments crossbred (Dominant White male x White Plymouth Rock female) chicks were used. In Experiments 1, 2 and 3, chicks were raised to one week of age on the reference ration and then equal numbers of male and female chicks were allotted to groups of 10 chicks each,

according to the method described by McKittrick (1947). In Experiment 4, day-old chicks were distributed into groups of 10 chicks each.

Also, in all experiments, each experimental ration was fed to two duplicate lots from either 0 to 42 or 7 to 42 days of age and to two other duplicate lots of chicks from 21 to 42 days of age. Chicks receiving the experimental rations from 21 to 42 days of age were maintained on the reference ration from 0 to 21 days. The feeding and management of the chicks have been described previously (see Part I, Experimental).

Excreta were collected at 24 hour intervals at 26, 27 and 28 days and again at 40, 41 and 42 days of age. Chromic oxide was incorporated in all rations as an index substance in order to eliminate the need for quantitative collection of excreta and quantitative measurement of feed intake. The methods for processing excreta and for conducting chemical analyses for moisture, nitrogen, combustible energy and for computing metabolizable energy from these data have been described previously (Hill and Anderson, 1958; Hill et al., 1960).

On completion of each of the experiments, all pullets in each group were killed, both thyroid glands were removed and thyroid-to-body-weight ratios were calculated as mg thyroid per 100 grams body weight.

Results and Discussion

Summarized in Table 5 (Experiment 1) are data showing the growth and thyroid size of chicks fed the soybean meal reference ration supplemented with 0, 0.045 and 0.090% of (±)-5-vinyl-2-oxazolidinethione (± OT). Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the growth data showed that the addition of 0.045 and 0.090% ± OT to the ration at seven days of age caused a significant ($P < 0.05$) and progressive decrease in rate of growth and a significant ($P < 0.05$) and progressive increase in thyroid size suggesting some degree of hypothyroidism. However, when the addition of these levels of ± OT was delayed to 21 days of age, growth rate was not depressed even at the highest level of supplementation (0.090%). Thyroid size was significantly ($P < 0.05$) increased at both levels of ± OT, but the increase at 0.090% ± OT was greater when supplementation was initiated at seven days of age than when it was initiated at 21 days of age. These results suggest that the older chicks, because of lower thyroxine requirement, were able to make thyroid adjustments and as a consequence did not suffer from hypothyroidism.

Also summarized in Table 5 (Experiments 2 and 3) are data showing the growth and thyroid size of chicks fed the reference ration and the rapeseed meal ration with and without added ground mustard seed, a source of myrosinase needed for the liberation of (-)-5-vinyl-2-oxazolidinethione (- OT) from its precursor in the rapeseed meal. Analysis of variance

TABLE 5. - Effect of goitrogens on growth and thyroid size of chicks

Exp.	Treatment	Gain, 1-6 weeks		Thyroid size, 6 weeks	
		Interval on ration, days		Interval on ration, days	
		7-42	21-42	7-42	21-42
		grams[1]		mg/100 g body wt[2]	
1	Reference	837[c,d]		10.9[a]	
	0.045%±OT[3]	780[b]	850[d]	82.4[b]	78.8[b]
	0.090%±OT	709[a]	815[c]	160.7[c]	98.5[b]
2	Reference	827[c]		12.6[a]	
	1% M.S.[4]	823[c]		13.6[a]	
	30% R.S.M.	710[a,b]	773[b,c]	19.5[a]	21.9[a]
	30% R.S.M. + 1% M.S.	702[a]	768[b,c]	75.9[c]	57.6[b]
3	Reference	870[b]		11.1[a]	
	30% R.S.M.	756[a]	794[a]	26.4[b]	20.4[b]
	30% R.S.M. + 1% M.S.	722[a]	738[a]	102.5[d]	72.2[c]
4	Reference	786[5,a]		10.2[a]	
	0.09% KSCN	761[5,a]	837[5,a]	12.3[a]	11.1[a]

[1]Values are averages of duplicate groups. Within an experiment, values without a common letter in their superscript are significantly different.

[2]Values are averages of 10 female chicks (5/replicate group).

[3](±)-5-vinyl-2-oxazolidinethione.

[4]Ground mustard seed added as a source of myrosinase for the liberation of (-)-5-vinyl-2-oxazolidinethione from its precursor in rapeseed meal.

[5]Gain from 0 day to 6 weeks of age.

and application of Duncan's multiple range test to the data in Experiments 2 and 3 showed that in contrast to Experiment 1, the level of - OT released by myrosinase (0.054 and 0.069% of the rations, respectively) in rations containing 30% rapeseed meal did not reduce growth rate significantly ($P > 0.05$) when compared against the groups that received rations containing only rapeseed meal, even though thyroid size was significantly increased ($P < 0.05$). As in Experiment 1, when the addition of mustard seed as a source of myrosinase was delayed to 21 days of age, the effect on thyroid enlargement was diminished. The failure of - OT to show any adverse effect on growth of chicks as compared to that produced by ± OT even at 0.069% in the ration suggest either reduced release of - OT from its precursor or a greater toxicity of ± OT.

Results of Experiment 4 show that supplementation of the reference ration with 0.090% KSCN had no significant effect ($P > 0.05$) on either growth or thyroid size. This suggests that sufficient iodine was present in the diet to permit the thyroid glands to compete successfully for iodine in the presence of thiocyanate.

Summarized in Table 6 (Experiment 1) are data showing the metabolizable energy content of the soybean meal reference ration supplemented with 0, 0.045 and 0.090% of ± OT. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data showed that the addition of 0.045% ± OT had no effect on metabolizable

TABLE 6. - Effect of goitrogens on the metabolizable energy value of rations for chicks

Exp.	Treatment	Interval on ration, days			
		7-28	21-28	7-42	21-42
		kcal/g		kcal/g	
1	Reference	3.24[1,b,c]		3.28[c]	
	0.045%±OT[2]	3.24[b,c]	3.24[b,c]	3.21[a,b]	3.28[c]
	0.090%±OT	3.16[a]	3.16[a]	3.20[a,b]	3.16[a]
2	Reference	3.24[d]		3.23[d]	
	1% M.S.[3]	3.20[d]		3.21[d]	
	30% R.S.M.	2.43[b,c]	2.38[a,b]	2.42[b,c]	2.32[a]
	30%R.S.M.+1%M.S.	2.51[c]	2.43[b,c]	2.43[b,c]	2.40[a,b]
3	Reference	3.20[d]		3.22[d]	
	30% R.S.M.	2.43[b,c]	2.38[a,b]	2.49[b,c]	2.52[c]
	30%R.S.M.+1%M.S.	2.43[b,c]	2.30[a]	2.45[b,c]	2.43[b,c]
4	Reference	3.13[a]		3.13[a]	
	0.090% KSCN	3.10[a]	3.08[a]	3.13[a]	3.12[a]

[1]Values are averages of duplicate groups. Within an experiment, values without a common letter in their superscript are significantly different.

[2](±)-5-vinyl-2-oxazolidienthione.

[3]Ground mustard seed added as a source of myrosinase for the liberation of (-)-5-vinyl-2-oxazolidinethione from its precursor in rapeseed meal.

energy. The addition of 0.090% ± OT reduced metabolizable energy slightly but significantly ($P < 0.05$) at both four and six weeks of age, irrespective of whether the experimental rations were fed from 7 or 21 days of age.

Also summarized in Table 6 (Experiments 2 and 3) are data showing the metabolizable energy content of the reference ration and the rapeseed meal ration with and without added ground mustard seed, a source of myrosinase. Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data in Experiments 2 and 3 showed that the - OT released (0.054 and 0.069% of the rations, respectively) had no significant effect ($P > 0.05$) on the metabolizable energy of the rations containing 30% rapeseed meal. Calculations indicate that the rapeseed meals in these rations contained 1.1 to 1.2 kcal of metabolizable energy per gram which is in close agreement with results reported previously (Part I, Table 3).

The levels of ± OT or - OT present in the test rations are much higher than would be encountered under practical feeding conditions since rapeseed meal normally accounts for only 10 to 15% of the ration and thus theoretically would provide a dietary level of only about 0.018 to 0.027% - OT if myrosinase was present to liberate - OT from its precursor. However, rapeseed meal contains no myrosinase and hence the only - OT released would be that released by myrosinase produced by intestinal microorganisms. Proof, that rapeseed meal contains no myrosinase, is verified by

the fact that the thyroid-to-body-weight ratios (Table 5, Experiments 2 and 3) of the chicks receiving the rations containing 30% rapeseed meal without added ground mustard seed is only about one-quarter that of the chicks receiving 30% rapeseed meal plus 1% ground mustard seed as a source of myrosinase. Hence, goitrogen(s) at the level present in rapeseed meal would not be expected to have any adverse effect on its metabolizable energy value.

Metabolizable energy values of the soybean meal reference ration with and without supplemental potassium thiocyanate are also summarized in Table 6 (Experiment 4). Analysis of variance showed that the addition of 0.090% KSCN did not affect the metabolizable energy value of the soybean meal reference ration at either four or six weeks of age, irrespective of whether the experimental rations were fed from 7 or 21 days of age.

In order to determine whether energy utilization was affected by the presence of goitrogens in the soybean meal reference ration and the rapeseed meal rations, caloric efficiencies were calculated during the 4th week of life after chicks had been fed the experimental rations from either 7 or 21 days of age. Results summarized in Table 7 indicate that the efficiency with which chicks utilized the soybean meal reference ration was not affected by the addition of either 0.045 or 0.090% ± OT irrespective of the time at which ± OT was incorporated in the ration (Experiment 1). Furthermore, results of Experiments 2 and 3 indicate that

TABLE 7. - Effect of goitrogens on caloric efficiency of chicks

Exp.	Treatment	Calories consumed per gram gain in fourth week	
		Interval on ration, days	
		7-28	21-28
		kcal/g gain[1]	
1	Reference	5.40[a]	
	0.045%±OT[2]	5.70[a]	5.40[a]
	0.090%±OT	5.01[a]	5.47[a]
2	Reference	5.53[c]	
	1% M.S.[3]	5.59[c]	
	30% R.S.M.	5.28[b,c]	4.53[a]
	30% R.S.M. + 1% M.S.	5.30[b,c]	4.83[a,b]
3	Reference	6.03[a]	
	30% R.S.M.	6.37[a]	5.50[a]
	30% R.S.M. + 1% M.S.	5.80[a]	5.23[a]
4	Reference	5.98[a]	
	0.09% KSCN	5.90[a]	5.72[a]

[1]See footnote 1, Table 5.

[2](±)-5-vinyl-2-oxazolidinethione.

[3]See footnote 4, Table 5.

the release of - OT in the rapeseed meal ration did not alter caloric efficiency even though thyroid size was markedly increased.

Results of previous experiments (Part I, Table 4) showed that chicks fed rations containing rapeseed meal prior to the 4th week of life utilized energy less efficiently during the 4th week of life than chicks in which the feeding of rapeseed meal was commenced at the beginning of the 4th week. It was suggested that this difference in caloric efficiency may have been due to changes in secretion of the thyroid gland. Results of the present experiment do not support this concept.

Summary

Studies were conducted to determine whether the relatively low metabolizable energy value of rapeseed meal could, in part, be due to the presence in rapeseed meal of thioglucosides which are converted by myrosinase to (-)-5-vinyl-2-oxazolidinethione (goitrin) and volatile isothiocyanates.

In Experiment 1, chicks were fed soybean meal-type rations supplemented with 0, 0.045 and 0.090% (±)-5-vinyl-2-oxazolidinethione. Metabolizable energy values of the rations were determined during the 4th and 6th week of life after the chicks had been fed the experimental rations from 7 to 21 days of age. Results showed that the addition of 0.090% but not 0.045% (±)-5-vinyl-2-oxazolidinethione reduced

metabolizable energy slightly at both four and six weeks of age, irrespective of whether the rations were fed from 7 or 21 days of age.

In Experiments 2 and 3, chicks were fed rations containing 30% rapeseed meal with and without added ground mustard seed as a source of myrosinase which is needed for the release of goitrin from its precursor in rapeseed meal. Results showed that the (-)-5-vinyl-2-oxazolidinethione released, calculated on the basis of analytical data at 0.054 to 0.069% - OT, had no effect on the metabolizable energy of the ration.

Results of Experiment 4 show that the addition of 0.09% KSCN had no effect on the metabolizable energy value of the soybean meal reference ration.

PART III

AVAILABILITY OF CARBOHYDRATE IN RAPESEED MEAL AND SOYBEAN MEAL

Review of Literature

Methods for Determining the Availability of Carbohydrates in Feedstuffs

Up to the present, three general methods have been used for estimating the carbohydrate in feedstuffs which is available for metabolism (hereafter referred to as available carbohydrate). These include: first, indirect estimation by difference, using the Weende chemical methods; second, biological determination of digestibility coefficient in which the Weende chemical methods are used to analyze the feces and ingested feed; third, direct chemical determination of carbohydrates either singly or by class.

The carbohydrate content of feed determined by difference using the Weende chemical methods, includes not only sugars and starch but small amounts of other complex carbohydrates as well as other substances such as organic acids and lignin (Browne, 1940). The method does not provide any sharp separation into chemical groups, but is useful because it is a simple procedure which makes a distinction between the more digestible carbohydrate (nitrogen-free extract) and the less digestible carbohydrate (crude fiber). However, several workers (Williams and Olmsted, 1935; Browne, 1940; Maynard, 1940) have criticized the Weende methods since considerable amounts of cellulose,

hemicellulose and lignin are dissolved during the acid and alkali treatments and are included in nitrogen-free extract. Hence, the partition of the carbohydrate of a feed into nitrogen-free extract and crude fiber is not an accurate measure of available carbohydrate.

The determination, in vivo, of the digestibility of carbohydrate is an improvement over the Weende chemical system for estimating available carbohydrate. Since the Weende methods are used for determining the carbohydrate in both the feed and feces, one might expect that errors resulting from the analyses of ingesta and excreta would tend to cancel each other. However, chemical changes in complex carbohydrates in the gastrointestinal tract mitigated perhaps by bacterial fermentation appear to render some of them soluble in dilute acid and alkali but still not absorbable. In this regard, numerous investigators (Hummel et al., 1943; Hoppert and Clark, 1945; Bolton, 1955) have found that although cellulose and hemicellulose are not usually classified as metabolizable by monogastrics, 20 to 80% of the crude fiber and pentosans in the diet are not recovered in the feces.

Several procedures have been proposed for the direct chemical determination of carbohydrates in feedstuffs. McCance and Widdowson (1940) assessed available carbohydrate by analyzing for starch, dextrin and di- and monosaccharides in feedstuffs with the underlying assumption that these are the only carbohydrates providing calories in significant amounts. This method has not been adopted because the

analytical procedures are complex and because insufficient information is available on the precision of the method to permit an assessment of its relative merit.

In 1956 a chemical method for the determination of available carbohydrate was proposed by Clegg. The simple sugars were first extracted with alcohol and the residual starches were then brought into solution with perchloric acid. The carbohydrate content of each of the aliquots was determined using anthrone. Available carbohydrate by this method, consisted of the sum of the soluble sugars and the residual starches.

The available carbohydrate content of a feed ingredient has also been determined chemically in terms of reducing sugars after enzymatic hydrolysis with Takadiastase (Bolton, 1960). Similar values for the available carbohydrate in cereals were obtained with these latter two methods. For example, Clegg (1956) found the available carbohydrate in wheat, oats and barley was 61.2, 42.0 and 53.9%, respectively. Comparative values reported by Bolton (1960) were 58.5, 38.4 and 53.9%, respectively.

More recently, Friedmann et al. (1967) described a procedure for determination of available carbohydrate in foodstuffs. In their method, the sample was hydrolysed by acid or enzyme or a combination of the two to convert the starch and dextrin to reducing sugars. The latter were then determined by ferricyanide reduction after clarification of the hydrolysate with zinc hydroxide.

Southgate (1969) also has proposed a scheme for determining the available carbohydrate content of food. In his scheme, the food was extracted with alcohol to dissolve the sugars and then the residual material was hydrolysed, using dilute sulfuric acid or the enzyme Takadiastase, to convert starch to glucose. The reducing sugars, thus obtained, were determined either in a single assay using anthrone or by separate assays for the individual sugars. Since it has been shown that acid hydrolysis results in loss of 3 to 4% of glucose and at least 50% of fructose (Davis and Daish, 1914), it may be assumed that methods involving acid hydrolysis will underestimate the amount of carbohydrate present. However, where enzymatic hydrolysis is involved, carbohydrate present may also be underestimated since in vitro enzymatic hydrolysis seldom goes to completion.

Available Carbohydrate in Rapeseed Meal and Soybean Meal

Data on the amount of available carbohydrate in rapeseed meal are limited. Hrdlicka et al. (1965) reported that the soluble sugar, starch and pentosans content of rapeseed meal was 3.2, 5.8 and 10.0%, respectively.

Information on the carbohydrates present in soybean meal has accumulated using some of the aforementioned methods. Bolton (1957) determined the sugar and starch content of soybean meal (49.0% protein) by first extracting the sugar with alcohol and then hydrolysing the residual material with Takadiastase. Using this method he found that soybean meal

contained 17.6% available carbohydrate. Subsequently, Bolton (1960) reported 12.6% available carbohydrate in soybean meal (44% protein) when a single assay was used after hydrolysing the whole sample with Takadiastase. From a survey of the literature Hardinge et al. (1965) reported that soybean meal contains 2.0% reducing sugars, 9.0% sucrose, 1.8% dextrin, 2.4% starch and 5.0% pentosans.

Studies at the University of Alberta

None of the methods described in the "Review of Literature" estimate the carbohydrate which has actually been absorbed and is available for metabolism. Recently, Renner and Elcombe (1964) and Brambila and Hill (1966) showed that the growth of chicks fed diets in which soybean fatty acids served as the sole source of non-protein energy was markedly increased by the addition of a dietary source of carbohydrate. This finding suggested that the available carbohydrate in rapeseed meal and soybean meal might be determined by comparing the growth response obtained when these meals served as the source of carbohydrate with the growth response obtained when the "carbohydrate-free" ration was supplemented with graded levels of glucose. The following studies were undertaken to attempt to determine the available carbohydrate content of rapeseed meal and of soybean meal by this means and to compare the values so obtained with those obtained using the chemical method of Clegg (1956).

Experimental

The composition of the "carbohydrate-free" ration employed in the bioassay is shown in Table 8. The ration was formulated to contain 15.4 kcal of metabolizable energy per gram protein, using the values 3.83 (Hill, 1962) and 8.65 kcal per gram (Renner, 1964) for the metabolizable energy of soybean protein (promine) and soybean fatty acids, respectively. At this ratio, protein is present in sufficient quantities to promote rapid growth but is not in excess (Renner, 1964; Renner and Elcombe, 1967). Rations containing glucose in amounts to supply 0.018, 0.035, 0.070 and 0.105 gram per gram soybean fatty acids were formulated from the "carbohydrate-free" ration by replacing the energy in soybean fatty acids by an equicaloric amount of a mixture of glucose and soybean fatty acids. Rations containing rapeseed meal or soybean meal as sources of carbohydrate were maintained isonitrogenous by reducing the amount of soybean protein in the "carbohydrate-free" ration, and isocaloric by adjusting the levels of soybean fatty acids in the rations. The levels of rapeseed meal and soybean meal used in the assay were calculated to contain less than the chick's requirement for carbohydrate which is approximately 0.05 gram glucose per gram soybean fatty acids. The metabolizable energy values of glucose, rapeseed meal and soybean meal used in formulating the rations were 3.64 (Anderson et al., 1958), 1.20 (Part I, Table 3) and 2.77 kcal per gram (Hill and Renner, 1960), respectively.

TABLE 8. - Composition of "carbohydrate-free" ration[1]

Ingredients	Amount
	%
Soybean protein[2]	33.57
Glycine	0.93
DL-methionine	1.19
Soybean oil	2.94
Vitamin mixture[3]	0.83
Mineral mixture[4]	8.07
Cellulose[5]	10.58
Soybean fatty acids[6]	41.41
Chromic oxide	0.44
Antioxidant (Ethoxyquin)	0.037

[1]Calculated metabolizable energy content of diet = 5.14 kcal/g.

[2]Promine, Central Soya, Chemurgy Division, Chicago 60639.

[3]Supplied per 100 grams ration: thiamine, 1.47 mg; riboflavin, 1.47 mg; calcium pantothenate, 5.89 mg; biotin, 0.06 mg; pyridoxine, 2.94 mg; niacin, 11.77 mg; folacin, 0.44 mg; menadione, 0.44 mg; vitamin B_{12}, 7.0 μg; choline chloride, 0.442 g; vitamin A, 1472 IU; vitamin D_3, 221 ICU; vitamin E, 4.9 IU; and aureomycin, 1.47 mg.

[4]Supplied in mg per 100 grams ration: $CaHPO_4$, 3164; $CaCO_3$, 2193; NaCl, 883; KH_2PO_4, 1369; $MgSO_4$, 356; KI, 0.43; $FeSO_4 \cdot 7H_2O$, 40.91; $CuSO_4 \cdot 5H_2O$, 2.30; $ZnCO_3$, 16.92; $CoCl_2 \cdot 6H_2O$, 0.25; $NaMoO_4 \cdot 2H_2O$, 1.22; Na_2SeO_3, 0.032; and $MnSO_4 \cdot H_2O$, 48.6.

[5]Solka Floc, S. W. - 40 - A, Brown Forest Products, Limited, Montreal, Quebec.

[6]Prepared from soybean oil (Renner and Elcombe, 1964).

Male crossbred chicks (Dominant White male x White Plymouth Rock female) were fed, during a pre-experimental period of one week, a "carbohydrate-free" ration in which non-protein energy was supplied by soybean oil. This ration was formulated from the "carbohydrate-free" ration (Table 8) by replacing 41.41 parts soybean fatty acids by 38.68 parts soybean oil. At the end of this period, duplicate groups of 10 chicks were allocated to each treatment on the basis of body weight and rate of gain using the procedure described by McKittrick (1947). The chicks were housed in electrically heated, thermostatically controlled battery brooders with raised wire screen floors in a temperature-controlled laboratory. Feed and water were supplied ad libitum. The chicks were weighed weekly and feed wastage was determined daily.

Standard growth response curves for glucose were obtained by plotting total weight gained by chicks during the two-week experimental period against the $\log_{10}$ (1+ gram glucose per gram soybean fatty acids). These response curves were used to calculate the carbohydrate content of rapeseed meal and soybean meal from weight gains obtained from these meals when they served as the source of carbohydrate.

Chemical assays for available carbohydrate in rapeseed meal and soybean meal were conducted using the method of Clegg (1956). In this method starch and soluble sugars were extracted and measured colorimetrically after the addition of anthrone. For comparative purposes nitrogen-

free extract was also determined. Proximate analyses were conducted using A.O.A.C. methods (1960).

The proximate composition of the rapeseed meals used in this study has been given in Table 1 (Part I). The soybean meal used contained 53.6% protein, 1.1% fat, 3.6% crude fiber, 6.5% ash and 35.2% nitrogen-free extract, moisture-free basis.

Results and Discussion

Standard growth response curves for three chick bioassays, obtained by plotting weight gains against dietary glucose level expressed as $\log_{10}$ (1+ gram glucose per gram soybean fatty acids) are shown in Figure 2. The metabolic requirement for carbohydrate determined by intersection of linear and plateau lines varied from 0.042 to 0.054 gram glucose per gram soybean fatty acids. Since the metabolic requirement for carbohydrate can be met from a wide variety of precursors, the requirement stated in the present study is only applicable under these conditions. These results confirm the finding of Renner and Elcombe (1964) that the requirement for maximum growth response is in the range of 0.035 to 0.105 gram glucose per gram fatty acids.

Analysis of variance and application of Duncan's multiple range test (Steel and Torrie, 1960) to the data on growth showed that in all three experiments the addition of glucose up to a level of 0.070 gram glucose per gram fatty acids resulted in progressive and significant increases in

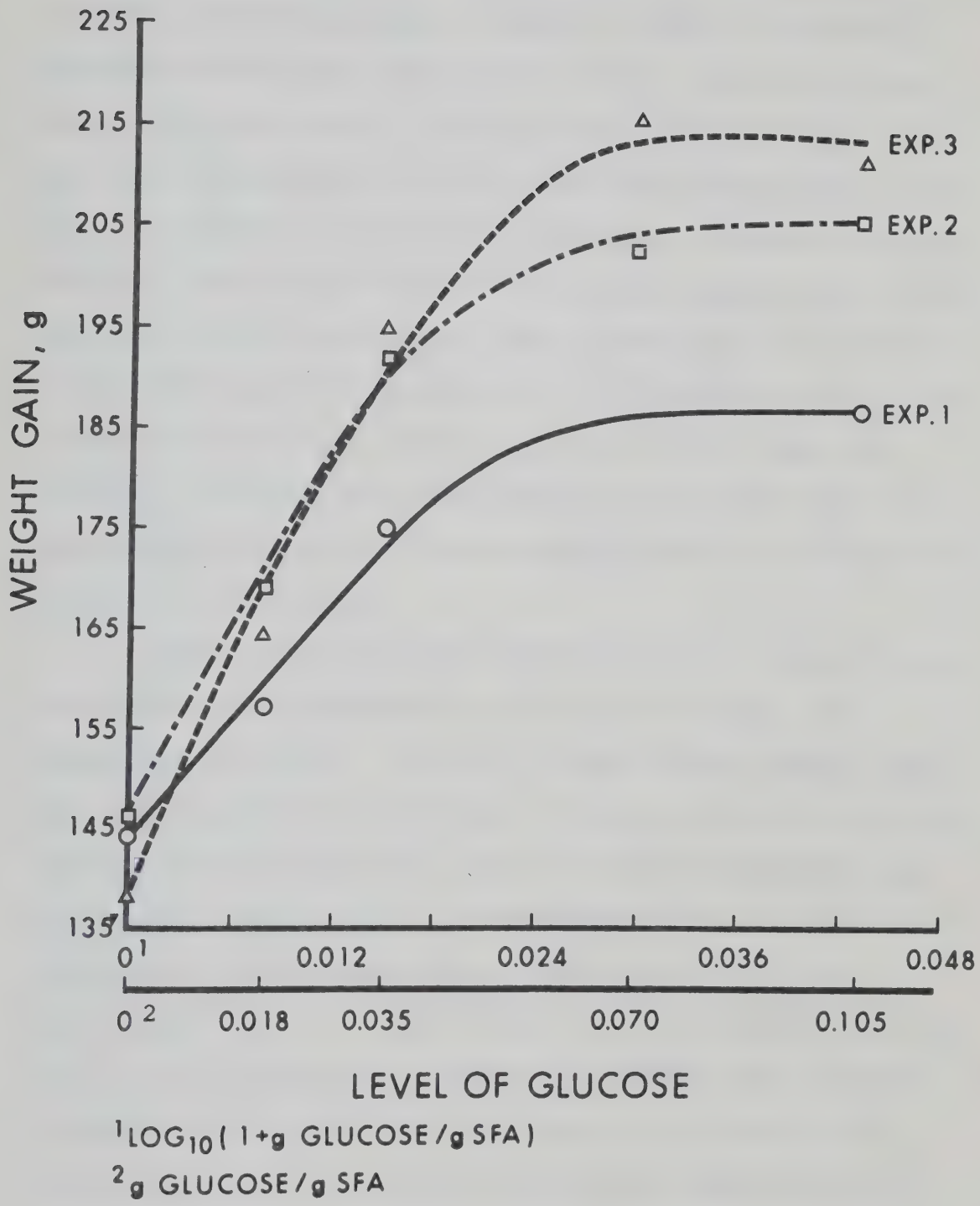


FIGURE 2. Growth response of chicks fed graded levels of glucose expressed as $\log_{10}$ (1+ g glucose/g soybean fatty acids)

growth. Supplementation with higher levels of glucose failed to cause additional growth response. Highly significant positive correlation coefficients (0.88, 0.88, 0.85) were obtained between level of carbohydrate and weight gain in all three experiments. Since growth was proportional to the level of glucose in the ration and since in previous studies (Renner, 1966) it was shown that fructose, galactose, xylose, sorbitol, dextrin and starch were as effective as glucose in stimulating growth of chicks fed "carbohydrate-free" rations, it was concluded that growth response could be used to determine the available carbohydrate in rapeseed meal and soybean meal even though carbohydrates other than glucose are present in these meals.

Data on the available carbohydrate in rapeseed meal and soybean meal determined by the chick bioassay are summarized in Table 9. As will be seen in the table, each meal was assayed at two or three levels, and each level was calculated to provide less than 0.05 gram carbohydrate per gram soybean fatty acids. The data show that the available carbohydrate in rapeseed meal #5 was 4.5 and 6.3% in Experiments 2 and 3, respectively, and for rapeseed meal #8 was 8.4% (Experiment 3). Values for soybean meal obtained in Experiments 1 and 2 were 14.0 and 14.2%, respectively. The variability in available carbohydrate observed within a given experiment is within the variation of biological experimentation.

Values for chemically available carbohydrate in nine

TABLE 9. - Available carbohydrate in rapeseed meal and soybean meal determined by the chick bioassay

Level and source of CHO	Exp. 1		Exp. 2		Exp. 3	
	Wt gain[1]	CHO[2]	Wt gain[1]	CHO[2]	Wt gain[1]	CHO[2]
g/g SFA	g	%	g	%	g	%
Soybean meal						
0.143			177	16.1		
0.175	162[3]	13.0				
0.214			187	14.5		
0.286			189	11.9		
0.350	181	15.1				
Average		14.0		14.2		
Rapeseed meal 5[4]						
0.250			161	4.8		
0.350					175	6.6
0.375			168	4.5		
0.437					178	5.9
0.500			174	4.4		
Average				4.5		6.3
Rapeseed meal 8[5]						
0.375					183	8.3
0.500					195	8.5
Average						8.4

[1]7-21 days of age.

[2]Available carbohydrate.

[3]Values are averages of duplicate groups of 10 chicks.

[4]Solvent processed.

[5]Prepress-solvent processed.

samples of rapeseed meal and one sample of soybean meal determined by the method of Clegg (1956) are summarized in Table 10. For comparative purposes values for nitrogen-free extract are also given. Calculations indicate that in rapeseed meal soluble sugars and starch make up about 40% of the nitrogen-free extract, whereas in soybean meal soluble sugars and starch comprise 67% of the nitrogen-free extract.

Comparison of the data summarized in Tables 9 and 10 shows that values for available carbohydrate obtained by Clegg's chemical method are higher than those obtained by the chick bioassay. This may be due to the presence in the extract of reducing compounds other than carbohydrate which react with the anthrone reagent. On the other hand, the difference could be due to the fact that the bioassay reflects not only the amount of these components, but also their absorbability. Furthermore, the bioassay would also underestimate available carbohydrate if any of the carbohydrates were converted to volatile fatty acids through fermentation in either the crop or caecum. The extent to which carbohydrates are fermented in the crop of the chick is unknown but may be insignificant because of the relatively rapid rate of passage of food through its gastrointestinal tract.

Bolton (1957), from results of absorbability trials conducted with adult chickens, concluded that the sugars and starch in soybean meal determined chemically were 100% absorbable. He reported that the level of sugars and starch

TABLE 10. - Carbohydrate content of rapeseed meal and soybean meal determined by the chemical method[1]

	Nitrogen-free extract	Soluble sugars	Starch[2]	Available CHO[3]
	%	%	%	%
Rapeseed meal #1	34.3	11.5	2.3	13.8
Rapeseed meal #2	36.6	10.8	3.3	14.1
Rapeseed meal #3	33.9	11.0	2.2	13.2
Rapeseed meal #4	36.1	12.4	2.2	14.6
Rapeseed meal #5	34.2	12.0	2.4	14.4
Rapeseed meal #6	35.2	11.2	2.2	13.4
Rapeseed meal #7	34.2	11.5	2.1	13.6
Rapeseed meal #8	35.3	12.4	3.2	15.6
Rapeseed meal #9	36.1	11.8	2.7	14.5
Averages	35.1	11.6	2.5	14.1
Soybean meal	35.3	18.6	5.0	23.6

[1]Values are expressed on a dry matter basis.

[2]Expressed as soluble sugars.

[3]Soluble sugars plus starch.

in soybean meal containing 49% protein on a dry matter basis was 17.6%. Subsequently, Bolton (1960) found that the available carbohydrate in soybean meal (44% protein) was 12.6% on a dry matter basis. These results are in general agreement with results obtained in the present study using the chick bioassay.

The metabolizable energy value of rapeseed meal was 1,203 kcal per kilogram (Part I, Table 3) for four week old chickens fed rations containing 30% rapeseed meal. This is approximately half the metabolizable energy content of soybean meal (56.4% protein) which according to Hill and Renner (1960) is 2,770 kcal per kilogram. The finding that the level of available carbohydrate in rapeseed meal is lower than in soybean meal helps to explain why the metabolizable energy value of rapeseed meal is low. Calculation, using results of the chick bioassay and assuming an energy value of 4.00 kcal per gram for the carbohydrate in the two meals, indicate that the difference in amount of available carbohydrate in rapeseed meal and soybean meal accounts for 290 kcal per kilogram of the total difference in metabolizable energy value (1,570 kcal per kilogram) of these two feedstuffs.

Summary

Available carbohydrate in two samples of rapeseed meal and one sample of soybean meal was determined using a chemical method and a chick bioassay. Results of the chemical

assay showed that the total soluble sugars and starch in the two samples of rapeseed meal and the one sample of soybean meal were 15.0 and 23.6%, respectively. Results of the bioassay showed that the available carbohydrate in the same samples of rapeseed meal and soybean meal was 6.9% and 14.1%, respectively. The values obtained by bioassay are lower than those obtained by chemical analysis, which may be due to the fact that the bioassay reflects not only the amount of available carbohydrate but also its absorbability. The difference in amount of available carbohydrate in rapeseed meal and soybean meal was found to account for 290 fewer kcal of metabolizable energy per kilogram in rapeseed meal than in soybean meal.

ABSORBABILITY OF PROTEIN IN RAPESEED MEAL BY GROWING CHICKENS AND LAYING HENS

Review of Literature

Methods for Determining Protein Absorbability

Determination of the absorbability of protein by avian species is difficult because urine and feces are excreted together. Methods which have been used to separate fecal nitrogen from urinary nitrogen include: (1) mechanical separation, (2) chemical separation and (3) surgical alteration so that urine and feces are excreted separately.

Mechanical separation of the "white cap" of urate from avian excreta was attempted by Heller et al. (1930). Ekman et al. (1949) state that since a considerable amount of urinary nitrogen in aqueous solution saturated the feces, mechanical removal of the "white cap" cannot lead to quantitative separation of urinary nitrogen from fecal nitrogen.

Methods for chemical separation of fecal nitrogen from urinary nitrogen are based on two different principles. One of these involves direct determination of fecal nitrogen, while the other depends on indirect estimation of fecal nitrogen by subtracting urinary nitrogen from total nitrogen in the excreta (Ekman et al., 1949).

The direct determination of fecal nitrogen involves dissolving the uric acid in the feces and then precipitating the fecal nitrogen which is composed mainly of protein by the addition of a protein-precipitating reagent. Difficulties encountered include that of dissolving the uric acid without

altering the protein and then precipitating the protein without precipitating the uric acid.

In estimating fecal nitrogen by difference from determinations of urinary nitrogen and total nitrogen, difficulties are encountered because urinary nitrogen arises from a heterogenous mixture of nitrogen containing compounds consisting of uric acid, urea, ammonia, creatine, creatinine, amino acids and some protein. It is customary to determine either uric acid or uric acid and ammonia and calculate urinary nitrogen on the assumption that urinary nitrogen has a constant composition. However, Davis (1927), Ekman (1948) and O'Dell et al. (1960) have shown that uric acid nitrogen may constitute from 60 to 80% of total urinary nitrogen. The composition of urinary nitrogen is also known to depend on the type of dietary protein and other dietary factors (Creek and Vasaitis, 1961; Richardson et al., 1968; Teekell et al., 1968). Thus, it is not surprising to find that protein absorbabilities determined chemically using direct and indirect methods often do not agree.

Recently, surgical techniques for exteriorizing the large intestine of mature birds have been perfected (Ariyoshi and Morimoto, 1956; Richardson et al., 1960) which permit collection of urine and feces separately. One criticism of this method is that the birds may not be physiologically normal after the operation, although it has been shown that laying hens, after operation, regain lost body weight (Ariyoshi and Morimoto, 1956) and attain normal egg production

(Richardson et al., 1957).

A fourth method for estimating protein absorption which might be used depends on the fact that the upper part of the small intestine is the site of most active absorption. Carroll et al. (1952), using rats, found that by the time the terminal fifth of small intestine was reached 91% of the protein in soybean meal which could be absorbed, was actually absorbed. Subsequently, Carroll et al. (1953), in studies with rats, found that 98% of the absorbable protein in soybean meal was absorbed before the foodstuff passed through the fifth segment. Recently, Imondi and Bird (1965) and Bird (1968) studied the absorbability of protein in a practical-type broiler ration using the nitrogen content of the material in various segments of the chick's small intestine as a measure of unabsorbed nitrogen. They found that 72 to 90% of the nitrogen reaching the duodenum was absorbed in the upper half of the jejunum. By the time the feed had passed the lower end of the ileum, cumulative nitrogen absorption was 95%. These studies showed that the absorption of dietary nitrogen was almost completed by the time the feed reached the posterior end of the small intestine. As a consequence of these studies, it should be possible to estimate protein absorbability by comparing the amount of unabsorbed nitrogen in the terminal fifth of the small intestine with the amount present in the ration. Renner (1965) estimated fat absorbability in this manner using chickens. She found that by the time the terminal fifth segment of small intestine was reached 90% of

the tallow, 91% of the lard and 98% of the soybean oil to be absorbed, was absorbed.

Absorbability of Protein in Rapeseed Meal

Information on the absorbability of protein in rapeseed meal for poultry is limited. Rutkowski et al. (1965), employing the chemical method of Ekman et al. (1949) for separation of urinary and fecal nitrogen, reported that the apparent absorbability of protein in a ration containing 0 and 8.5% of rapeseed meal was 85.8 and 77.3%, respectively, for 12 week old chickens. These results indicated that the protein in rapeseed meal was less well absorbed than the mixture of proteins which it replaced. Kubota and Morimoto (1965), using colostomized hens, found that the absorbability of protein in one sample of rapeseed meal was 72.6%. In their study the meal was the sole source of dietary protein.

Studies on the absorbability of protein in rations containing rapeseed meal have been reported for other monogastric animals. Hussar and Bowland (1959) reported that the addition of 10% rapeseed meal to a ration as a replacement for soybean meal reduced the absorbability of dietary protein by rats but not by pigs. Subsequently, Manns and Bowland (1963) found that replacement of 50 or 100% of dietary soybean meal with rapeseed meal depressed the absorbability of protein by rats and pigs. Recently, Drouliscos and Bowland (1969) reported that the apparent and true protein absorbability of rapeseed meal was 74 and 79%, respectively, for weanling rats

when the meal served as the sole source of protein. The corresponding values for mature rats were 79 and 83%, respectively.

Studies at the University of Alberta

The following studies were undertaken to determine the absorbability of the protein in rapeseed meal by growing chickens and laying hens. For comparative purposes similar studies were also conducted on soybean meal. It was hoped that such information might help to explain why the chick and hen have limited ability to utilize the energy in rapeseed meal.

Experimental

Four experiments were conducted. In Experiments 1 and 2 the absorption, by chicks and laying hens, of nitrogen from rations containing rapeseed meal or soybean meal as the sole source of protein was studied using the nitrogen remaining in the terminal fifth segment of the small intestine as the measure of unabsorbed nitrogen. In Experiments 3 and 4 absorbability of nitrogen from rations containing rapeseed meal or soybean meal as the sole source of protein was determined using colostomized hens. In this instance the nitrogen remaining in the urine-free feces was used as the measure of unabsorbed nitrogen.

The composition of the rations used in the study are shown in Table 11. Rapeseed meal or soybean meal

TABLE 11. - Composition of experimental rations

	Fed to chicks				Fed to hens				
Ration number	1	2	3	4	5	6	7	8	9
Ingredients	%	%	%	%	%	%	%	%	%
S.B.M.[1] #1	42.68			42.68	32.53				
S.B.M. #2								32.3	
R.S.M.[2] #1		49.53				37.58			
R.S.M. #2			52.83	30.0			40.25		
R.S.M. #3									39.56
Soybean oil	2.0	15.0	15.50	2.0	2.0	8.29	9.10	2.0	9.09
Glucose (Cerelose)	47.30	27.45	23.65	17.30					
Sucrose					54.64	43.30	39.82	54.87	40.52
Glycine	1.00	1.00	1.00	1.00					
DL-methionine	0.50	0.50	0.50	0.50	0.10	0.10	0.10	0.10	0.10
Mineral mixture	4.91[3]	4.91[3]	4.91[3]	4.91[3]	9.40[4]	9.40[4]	9.40[4]	9.40[4]	9.40[4]
Vitamin mixture	0.58[5]	0.58[5]	0.58[5]	0.58[5]	0.32[6]	0.32[6]	0.32[6]	0.32[6]	0.32[6]
Antioxidant (Ethoxyquin)	0.025	0.025	0.025	0.025	0.01	0.01	0.01	0.01	0.01
Chromic oxide mixture[7]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 11. continued

[1]Soybean meal.

[2]Rapeseed meal.

[3]Supplied in milligrams per 100 grams ration: $CaHPO_4$, 2,600; $CaCO_3$, 1,300; NaCl, 600; K_2HPO_4, 220; $MgSO_4$, 115; $FeSO_4 \cdot 7H_2O$, 28; $MnSO_4 \cdot H_2O$, 33.5; $ZnCO_3$, 9.7; $CuSO_4 \cdot 5H_2O$, 0.78; KI, 0.29; and Na_2SeO_3, 0.022.

[4]Supplied in milligrams per 100 grams ration: $CaHPO_4$, 4,000; $CaCO_3$, 4,500; NaCl, 500; K_2HPO_4, 220; $MgSO_4$, 120; $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 16.8; $ZnCO_3$, 9.25; $CuSO_4 \cdot 5H_2O$, 1.5, KI, 0.33; and Na_2SeO_3, 0.02.

[5]Supplied per 100 grams ration: thiamine, 1.0 mg; riboflavin, 1.0 mg; calcium pantothenate, 4.0 mg; biotin, 0.04 mg; pyridoxine, 2.0 mg; niacin, 8.0 mg; folacin, 0.3 mg; menadione, 0.3 mg; vitamin B_{12}, 0.5 μg; choline chloride, 0.3 g; vitamin A, 1,000 IU, vitamin D_3, 150 ICU; vitamin E, 3.3 IU; and aureomycin, 1.0 mg.

[6]Supplied per 100 grams ration: thiamine, 1.0 mg; riboflavin, 2.0 mg; calcium pantothenate, 5.0 mg; niacin, 15.0 mg; folacin, 0.2 mg; pyridoxine, 1.5 mg; biotin, 0.05 mg; vitamin A, 1,000 IU; vitamin D_3, 75 ICU; vitamin E, 1.1 IU; and aureomycin, 1.0 mg.

[7]Contained: 30% chromic oxide, 70% wheat flour.

appropriately supplemented with methionine and glycine served as the sole source of nitrogen. Non-protein energy was provided by glucose and soybean oil, in the case of the rations for chicks, and by sucrose and soybean oil, in the case of the rations for hens. The paired rations were isonitrogenous and were formulated to contain 14.1 kcal of metabolizable energy per gram protein for chicks, and 18.8 kcal per gram protein for hens. Chromic oxide was incorporated in all rations at a level of approximately 0.3% as an index substance.

In the absorbability studies with chicks, Dominant White x White Plymouth Rock male and female chicks were raised to one week of age on a reference ration (Part 1, Table 2). At the end of the pre-experimental period, 5 male and 5 female chicks were allotted to groups according to the method described by McKittrick (1947). In Experiment 1, triplicate groups of chicks were fed rations 1 and 2 (Table 11), while in Experiment 2, triplicate groups of chicks were fed rations 1, 3 and 4. The experimental rations were fed from 7 to 42 days of age. The feeding and management procedures followed were similar to those described previously (Part I, Experimental).

In order that metabolizable energy could be determined, excreta were collected from each group of chicks on the 26th, 27th and 28th day and again on the 40th, 41st and 42nd day. The three-day pooled collections for each group were maintained in the frozen state until processed.

The methods for processing excreta and for conducting chemical analyses for moisture, nitrogen, combustible energy and chromic oxide were similar to those described by Hill and Anderson (1958) and Hill et al. (1960). The metabolizable energy values for rapeseed meal and soybean meal were calculated from the determined metabolizable energy values of the respective rations by subtracting the energy contributed by the glucose and soybean oil included in the rations. In these calcualtions glucose and soybean oil were assigned metabolizable energy values of 3.64 (Anderson et al., 1958) and 9.21 (Renner and Elcombe, 1964) kcal per gram, respectively.

At six weeks of age, the chicks were killed with chloroform and the digestive tracts were removed immediately. The small intestine from the proximal end of the duodenum to the junction of the caeca and colon, was divided into five segments of equal length. The contents of the terminal fifth segment of the small intestine were removed using distilled water and gentle pressure. This was done immediately after killing to avoid, in so far as possible, shedding of epithelial tissue into the gut. The intestinal contents thus obtained were freeze-dried and analyzed for chromium and nitrogen using the methods described by Hill and Anderson (1958). The absorbability of nitrogen was determined by comparing the ratio of nitrogen to chromic oxide in the feed, to the ratio of nitrogen to chromic oxide in the contents of the terminal fifth of the small intestine.

The thyroid glands of the female chicks in each group

were removed and thyroid-to-body-weight ratios were calculated as mg thyroid per 100 grams body weight.

As previously indicated, in Experiments 1 and 2 the nitrogen absorption of hens fed rations containing rapeseed meal or soybean meal was also studied using the nitrogen remaining in the terminal fifth of the small intestine as a measure of unabsorbed nitrogen. In Experiment 1, duplicate groups of 10 Single Comb White Leghorn hens (19 months old) were fed rations 5 and 6 (Table 11) for two weeks, while in Experiment 2, triplicate groups of 10 hens were fed rations 5 and 7 for four weeks. In both experiments nitrogen absorption was determined at the end of the feeding period by the terminal fifth segment technique previously described. In addition, in Experiment 2 fecal collections were made on the 26th, 27th and 28th day of the experiment to permit metabolizable energy determinations.

In Experiments 3 and 4, nitrogen absorption was determined using colostomized hens. Colostomy was performed by the method of Ariyoshi and Morimoto (1956). After surgery, the hens were maintained on a low fibre, high energy ration for four weeks to permit them to recover from the operation. In Experiment 3, rations 8 and 9 (Table 11) were fed to groups of five colostomized hens for 19 days, while in Experiment 4, these rations were fed to groups of five colostomized hens for 33 days. Fecal collections were made at 12 hour intervals on five consecutive days after the hens had been fed the experimental rations for two or four

weeks. This was done by attaching a polyethylene bag around the colostomized anus according to the procedure described by Rothchild (1947). The five-day pooled collections for each hen were freeze-dried.

Digestible energy values for rations 8 and 9 were determined by a procedure similar to that used for the determination of metabolizable energy. The digestible energy contents of rapeseed meal and soybean meal were calculated from the determined digestible energy values of the respective rations by subtracting the energy contributed by the sucrose and soybean oil included in the rations. The sucrose and soybean oil, used in the rations for hens, were assumed to have digestible energy values equal to their metabolizable energy values of 3.80 (Hill, 1962) and 9.21 kcal (Renner and Elcombe, 1964) per gram, respectively.

The proximate composition of the meals (Table 12) was determined using A.O.A.C. methods (1960). Oxazolidinethione and isothiocyanate contents of rapeseed meal (Table 12) were determined using the methods of Astwood et al. (1949) and Wetter (1955, 1957).

Results and Discussion

Summarized in Table 13 are data showing the apparent absorbability of nitrogen in rapeseed meal and soybean meal for six week old chickens and hens. Results based on the determination of unabsorbed nitrogen in the terminal fifth of the small intestine (Experiments 1 and 2) showed that the

TABLE 12. - Composition of rapeseed meals and soybean meals[1]

	Moisture	Protein (Nx6.25)	Fat	Fibre	Ash	Isothio-cyanate	Oxazolidine-thione
	%	%	%	%	%	mg/g	mg/g
R.S.M.[2] #1	8.4	39.2	2.8	11.3	5.6	2.10	7.73
R.S.M. #2	10.7	35.5	2.4	13.4	6.5	2.06	2.55
R.S.M. #3	7.3	37.7	2.2	12.3	6.4	1.85	2.78
S.B.M.[3] #1	10.8	43.9	1.8	5.2	6.0		
S.B.M. #2	6.9	46.1	1.4	6.5	5.9		

[1]Values are expressed on an air-dry basis.

[2]Rapeseed meal.

[3]Soybean meal.

TABLE 13. - Absorbability of the protein in rapeseed meal and soybean meal by chicks and laying hens when the respective meal served as the sole source of protein

Exp.	Source of protein	Apparent nitrogen absorption by terminal fifth segment method		Apparent nitrogen absorption by colostomy method
		Chicks	Hens	Hens
		%	%	%
1	R.S.M.[1] #1	80.1[3]	62.8[4]	
	S.B.M.[2] #1	85.8[3]	69.3[4]	
2	R.S.M. #2	79.7[3]	67.2[3]	
	S.B.M. #1	84.9[3]	72.0[3]	
3	R.S.M. #3			72.3[5]
	S.B.M. #2			80.0[5]
4	R.S.M. #3			76.9[5]
	S.B.M. #2			81.6[5]

[1]Rapeseed meal.

[2]Soybean meal.

[3]Values are the average of triplicate groups each containing 10 chicks or 10 hens.

[4]Values are the average of duplicate groups each containing 10 hens.

[5]Values are the average of 5 hens.

absorbabilities of nitrogen for two samples of rapeseed meal and one sample of soybean meal were 79.9 and 85.4%, respectively, for chicks, and 65.0 and 70.6%, respectively, for hens. Analyses of variance (Steel and Torrie, 1960) showed that in both chicks and hens the apparent absorbability of nitrogen in rapeseed meal was lower than that of soybean meal ($P < 0.05$). The author is unable to explain why the apparent absorption values for hens are lower than for chicks. If contamination of the contents of the terminal fifth of the small intestine with cellular debris was greater for hens than for chicks an explanation, however, could be suggested.

Also summarized in Table 13 are values for the absorbability of nitrogen in rapeseed meal and soybean meal obtained using colostomized hens. In Experiments 3 and 4, the absorption of nitrogen was lower when rapeseed meal served as the sole source of protein than when soybean meal served as the source of protein. Prolongation of the experimental period to four weeks in Experiment 4 was found to have no effect on apparent absorbability of the nitrogen in the rations containing either rapeseed meal or soybean meal. Values obtained after four weeks on experiment were 72.8 and 82.2%, respectively, compared to values of 76.9 and 81.6%, respectively, obtained after only two weeks on experiment.

Results of the present studies with colostomized hens on the apparent absorbability of nitrogen from rapeseed meal (74.6%) are in close agreement with the value of 72.6% reported by Kubota and Morimoto (1965). The average value

for soybean meal of 80.8% obtained in this study with colostomized hens is slightly lower than that of 88.6% obtained by Nitsan (1965) and the value of 85.7% reported by Nesheim and Garlich (1966).

The reason why the absorbability of nitrogen in rapeseed meal is lower than in soybean meal is not known. A possible explanation is that since rapeseed meal is higher in fiber more protein may be surrounded by indigestible carbohydrate, thus rendering it unavailable to the action of proteolytic enzymes. That the reduced nitrogen absorbability is not due to the goitrogen, (-)-5-vinyl-2-oxazolidinethione, in rapeseed meal is evident from the previous finding (Part II, Table 6) that (-)-5-vinyl-2-oxazolidinethione does not affect metabolizable energy.

Metabolizable energy values of rapeseed meal and soybean meal for four week old chicks, six week old chicks and laying hens are given in Table 14. The data show that when these meals served as the sole source of nitrogen the average metabolizable energy value of rapeseed meal was 1,880, 1,865 and 1,800 kcal per kilogram, respectively, for four week old chicks, six week old chicks and laying hens. The corresponding values for soybean meal were 2,770, 2,625 and 2,300 kcal per kilogram, respectively. The finding that rapeseed meal has a lower metabolizable energy than soybean meal is partially explained by the finding that both chicks and hens absorb a lower percentage of the nitrogen in rapeseed meal than in soybean meal.

TABLE 14. - Energy value of rapeseed meal and soybean meal for chicks and hens when the respective meal served as the sole source of protein

Exp.	Source of protein	Metabolizable energy[1,2]			Digestible energy[3]
		Chicks		Hens	Hens
		4 wk	6 wk		
		kcal/kg	kcal/kg	kcal/kg	kcal/kg
1	R.S.M.[4] #1	1,940	1,910		
	S.B.M.[5] #1	2,790	2,590		
2	R.S.M. #2	1,820	1,820	1,800	
	S.B.M. #1	2,750	2,660	2,300	
3	R.S.M. #3				1,820
	S.B.M. #2				2,390
4	R.S.M. #3				2,030
	S.B.M. #2				2,660

[1]Values are expressed on a dry matter basis.

[2]See footnote 3, Table 13.

[3]See footnote 5, Table 13.

[4]Rapeseed meal.

[5]Soybean meal.

Also presented in Table 14 are data showing the digestible energy of rapeseed meal and soybean meal determined using colostomized hens. The average digestible energy content of rapeseed meal and soybean meal for hens was 1,925 and 2,525 kcal per kilogram, respectively. These values are slightly higher than the corresponding metabolizable energy values, which is to be expected, since they do not take into account energy lost in the urine, whereas, metabolizable energy values do take urinary energy losses into account. Thus, it would appear that colostomizing the hens did not affect their utilization of nutrients.

Summarized in Table 15 are data showing the absorbability of nitrogen and the metabolizable energy values of rapeseed meal #2 obtained when chicks were fed a ration (Table 11, ration 4) in which 30 parts rapeseed meal was substituted, weight for weight, for glucose in a nutritionally complete ration containing soybean meal (Table 11, ration 1). In calculating these values it was assumed that rapeseed meal does not affect the absorbability of the nitrogen or the metabolizable energy of other dietary ingredients. For comparative purposes, values obtained when rapeseed meal served as the sole source of protein (Tables 13 and 14) are also included in Table 15. The data show that the apparent absorbability of nitrogen and the metabolizable energy content of rapeseed meal were significantly lower ($P < 0.05$) when rapeseed meal was substituted, weight for weight, for glucose in a ration containing soybean meal rather than when it served

TABLE 15. - Effect of composition of ration on utilization of rapeseed meal by growing chickens

	Rapeseed Meal #2	
	Replacing 30 parts glucose in ration containing soybean meal	Serving as the sole source of protein
Apparent nitrogen absorption, %[1]	63.0	79.7
Metabolizable energy, kcal/kg[1,2]		
4 wk	1,090	1,820
6 wk	1,150	1,820

[1]See footnote 3, Table 13.

[2]Values are expressed on a dry matter basis.

as the sole source of protein.

The finding that the nitrogen in rapeseed meal has a higher absorbability, when incorporated as the sole source of protein in a ration of normal protein content, than when 30 parts of rapeseed meal was substituted, weight for weight, for glucose in a nutritionally complete ration based on soybean meal, was unexpected. The latter ration was high in protein; however, studies have shown that the chick has the ability to digest and absorb high levels of casein (Anderson, 1955) and soybean meal (Olson et al., 1961; Sibbald and Slinger, 1962).

This inability to utilize nitrogen in a high protein ration containing soybean meal and rapeseed meal explains why the metabolizable energy content of rapeseed meal varies with method of substitution. That this inability to utilize the nitrogen of rapeseed meal in a high protein ration containing soybean meal disappears as the chicken matures is evident from the fact that the metabolizable energy of rapeseed meal for hens is similar whether it serves as the sole source of protein (1,800 kcal/kg, Table 14) or whether it is incorporated in the ration, weight for weight, for 30 parts of sucrose (1,782 kcal/kg, Part I, Table 3). That the ability of the chicken to utilize certain feedstuffs increases with age has been reported previously. In this regard, Renner and Hill (1960b) showed that the ability of the chick to utilize tallow increases with age. In addition, Renner and Hill (1960a), and Hill and Renner (1963) found that hens were

better able to digest and absorb the energy in heat damaged soybean flakes than chicks.

When the data on protein absorbability are used to calculate the contributions which the protein in rapeseed meal and soybean meal make to metabolizable energy, the lower absorbability of the nitrogen in rapeseed meal together with its lower protein content accounts for rapeseed meal having 710 fewer kcal of metabolizable energy per kilogram than soybean meal when rapeseed meal serves as the sole source of protein and 1,006 kcal per kilogram less when rapeseed meal is incorporated at the expense of glucose in a ration containing soybean meal. These calculations assume that rapeseed meal and soybean meal contain 40.2 and 56.4% protein on a dry matter basis and that absorbed protein would yield 4.34 kcal metabolizable energy per gram. Results of previous experiments (Part III, Table 9) showed that the difference in amount of available carbohydrate in rapeseed meal and in soybean meal accounts for 290 kcal of the difference in metabolizable energy value of these two feedstuffs. Thus, the total difference in metabolizable energy which can be accounted for by difference in protein content, nitrogen absorbability and available carbohydrate is 1,000 kcal per kilogram when rapeseed meal serves as the sole source of protein and 1,296 kcal when rapeseed meal is incorporated, weight for weight, for glucose in a ration containing soybean meal. Actual differences found between the metabolizable energy content of soybean meal (2,770 kcal, Hill and Renner,

1960) and rapeseed meal as the sole source of protein (1,872 kcal, Table 14, chicks) and rapeseed meal substituted weight for weight for glucose (1,313 kcal, Part I, Table 3), respectively, are 898 and 1,457 kcal per kilogram.

Data on growth, thyroid size, caloric efficiency and protein efficiency are shown in Table 16. Analyses of variance of the growth data showed that the body weight of chicks fed rapeseed meal as the sole source of protein is slightly but significantly lower ($P < 0.05$) than that of chicks fed soybean meal in Experiment 1, while the difference was not significant in Experiment 2. Thyroid size was significantly larger, and calories consumed per gram gain were significantly higher in the groups fed the rapeseed meal containing ration when compared to those fed the soybean meal containing ration in Experiment 1. While the trends were similar in Experiment 2 the differences were not significant ($P < 0.05$). The data on weight gain per gram of digestible protein consumed suggest that the quality of the protein of rapeseed meal compares favorably with that of soybean meal.

Summary

Studies were conducted to determine the apparent absorbability of nitrogen in rapeseed meal by growing chickens and laying hens. For comparative purposes, similar studies were also done on soybean meal. Results based on the determination of unabsorbed nitrogen in the terminal fifth of the small intestine of six week old chickens fed rations in

TABLE 16. - Effect of rapeseed meal and soybean meal as protein sources on body weight, thyroid size, caloric efficiency and gain per gram of digestible protein consumed

Exp.	Source of protein	Body wt, 6 wk	Thyroid size, 6 wk	Calories consumed per g gain, 7-42 days	Gain per g digestible protein consumed, 7-42 days
		g	mg/100g body wt	kcal	g
1	R.S.M.[1] #1	845[3,a]	22.7[4,b]	6.51[3,b]	2.80[3,b]
	S.B.M.[2] #1	974[b]	11.0[a]	6.15[a]	2.61[a]
2	R.S.M. #2	922[a]	13.9[a]	6.47[a]	2.83[b]
	S.B.M. #1	971[a]	8.3[a]	6.45[a]	2.41[a]

[1]Rapeseed meal.

[2]Soybean meal.

[3]Values are the average of triplicate groups each containing 10 chicks. Within an experiment, values without a common letter in their superscript are significantly different.

[4]Values are the average of 15 female chicks (5/replicate group).

which either rapeseed meal or soybean meal served as the sole source of protein showed that the absorbability of nitrogen in rapeseed meal and soybean meal was 79.9 and 85.4%, respectively. Nitrogen absorbability was also found to be lower for rapeseed meal than for soybean meal when colostomized hens were fed rations in which rapeseed meal or soybean meal served as the sole source of protein.

Metabolizable energy values of rapeseed meal for four week old chicks, six week old chicks and laying hens, determined when rapeseed meal served as the sole source of dietary nitrogen were 1,880, 1,865 and 1,800 kcal per kilogram, respectively. Comparable values for four and six week old chicks obtained when rapeseed meal was substituted, weight for weight, for 30 parts of glucose in a soybean meal reference ration were 1,090 and 1,150 kcal per kilogram, respectively. The higher metabolizable energy values obtained, when rapeseed meal served as the sole source of protein, were due, at least in part, to the difference in the absorbability of nitrogen under the two feeding systems (79.7 vs 63.0%).

Calculations based on absorbabilities found for rapeseed meal (substituted for 30 parts of glucose) and for soybean meal (supplied as the sole source of protein) and on protein contents for rapeseed meal and soybean meal of 40.2 and 56.4% (dry matter basis), respectively, indicate that the lower absorbability of the protein in rapeseed meal and the lower protein content of rapeseed meal as compared to soybean meal account for 1,006 fewer kcal per kilogram of metabolizable energy in rapeseed meal than in soybean meal.

Results of the foregoing studies have shown that the metabolizable energy value of rapeseed meal for growing chickens and laying hens is low when compared to soybean meal and other oil seed meals. Factors known to contribute to the low metabolizable energy value of rapeseed meal are its low protein content and high fiber content. In the present studies, other factors which have been found to contribute to this low metabolizable energy value include the availability of its carbohydrate and the absorbability of its protein. In the case of the growing chicken, the studies have also shown that the metabolizable energy content of rapeseed meal is higher when rapeseed meal serves as the sole source of dietary protein rather than when it is substituted weight for weight for glucose at a level of 30% in a reference ration containing soybean meal. In addition, it has been shown that the thioglucosides present in rapeseed meal do not contribute to its low metabolizable energy value even when the myrosinase that is required for the breakdown of the thioglucosides to (-)-5-vinyl-2-oxazolidinethione and volatile isothiocyanates is supplied from an exogenous source.

Consideration of the results of these studies suggest a number of ways by which the metabolizable energy value of rapeseed meal might be improved. These are outlined in the following paragraphs.

The metabolizable energy content of rapeseed meal could be increased if ways were found to increase its protein

content and decrease its fiber content. It would be ideal if this could be accomplished by the plant geneticist, however, another alternative would be to change the composition of rapeseed meal by mechanical removal of the bulk of the hull from the seed. Youngs (1967) has reported that this has been accomplished on a laboratory scale prior to oil extraction by passing the seeds through cracking rolls and separating the hulls from the meats by aspiration. In the case of Tanka rapeseed, he reported that the protein content of the resultant meal was increased from 44.7 to 53.6% and the crude fiber reduced from 11.8 to 3%. The effect that this might have on the metabolizable energy value of the meal was not studied.

The possibility also exists that digestion and absorption might be improved by supplementation of rations containing rapeseed meal with enzymes. In this regard, it should be noted that although rapeseed meal, on the average, contained 35.1% nitrogen-free extract it only contained 6.9% available carbohydrate. Thus, only 20% of the nitrogen-free extract was absorbed. In contrast, 79.9% of the protein in rapeseed meal was absorbed. These findings indicate that attempts at increasing metabolizable energy should be directed at increasing absorption of the nitrogen-free extract. The choice of enzyme or enzymes would be dependent on the carbohydrates present in the nitrogen-free extract. Since, at the present time, information on the carbohydrates in rapeseed meal is limited, studies might first be conducted

to determine both the kinds and amounts of the carbohydrates that occur in rapeseed meal.

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